HEART RATE AS A MONITOR FOR METABOLIC RATE IN CAPTIVE JUVENILE STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)

by

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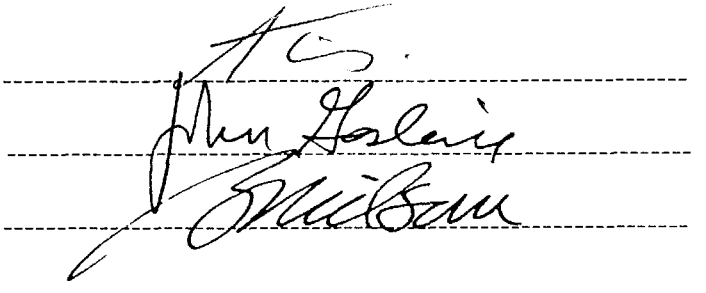

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ABSTRACT

The potential use of heart rate to monitor energy expenditure in free-ranging Steller sea lions (*Eumetopias jubatus*) was investigated by establishing whether a relationship exists between heart rate (f_H) and oxygen consumption ($\dot{V}O_2$) in captive sea lions while swimming and resting.

Four trained Steller sea lions (2 males and 2 females; mass 87.4 -194.4 kg; ages 16 months–3 years) were equipped with a datalogger and two dorsal electrodes to record ECG (from which f_H was calculated). Four styles of electrodes were developed and tested before selecting a final subcutaneous design. $\dot{V}O_2$ (measured with open-circuit respirometry) was simultaneously recorded while the previously-fasted animal was at rest within an enclosed dry metabolic chamber or while it swam in an enclosed swim mill against water currents of various speeds (0-1.5 $m \cdot s^{-1}$). The mean regression equation describing the relationship between f_H ($beats \cdot min^{-1}$) and $\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-0.73}$) for all four animals was $\dot{V}O_2 = (0.68 f_H \pm 0.07$ s.e.) – (15.07 ± 6.20) ($r^2=0.72$, $p<0.01$).

The possibility that the $f_H/\dot{V}O_2$ relationship could be affected by digestion was investigated by feeding one of the male Steller sea lions either 6 or 12 kg of herring prior to entering the swim mill. $\dot{V}O_2$ increased over time after ingestion, while heart rate usually remained stable or decreased. The resulting relationship, $\dot{V}O_2 = (0.24 f_H \pm 0.03)$ – (18.49 ± 02.68) ($r^2=0.19$, $p<0.01$), differed significantly from the relationship derived while the animal was fasted, indicating that digestion may alter the relationship between f_H and $\dot{V}O_2$. Fasting and feeding intervals must therefore be taken into account when

considering the use of $f_H/\dot{V}O_2$ relationships to estimate energy expenditure from heart rate of free-ranging sea lions.

The relationship demonstrated between f_H and $\dot{V}O_2$ while fasting suggests that heart rate can potentially be used to monitor energy consumption in free-ranging Steller sea lions. However, additional research should be conducted to further elucidate how the relationship is affected by such factors as digestion, sex, stress, and development.

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INTRODUCTION

Knowing how much energy an individual requires is fundamental to resolving a number of questions about marine mammals. These include determining how they have solved complex bioenergetic problems to successfully exploit their environment, and whether or not they compete with fisheries for prey (Markussen and Oritsland, 1991; Olesiuk, 1993; Rosen and Trites, 1997; Stenson et al., 1997; Trites et al., 1997; Boyd et al., 1999). Unfortunately, measuring energy expenditure of free-ranging individuals is difficult. Injecting doubly-labelled water and recording heart rate are two common techniques that have been applied.

The doubly-labelled water technique (DLW) (Lifson et al., 1955) involves injecting deuterium (D_2O) and $H_2{}^{18}O$ into the animal, allowing the isotopes to mix with the animal's CO_2 and body water pools, and subsequently measuring their rates of elimination (Costa, 1987; Bevan et al., 1995b). Oxygen-18 leaves the body as both water and respiratory CO_2, while deuterium leaves only as body water. The rate of CO_2 production can thus be estimated from the difference in the turnover rates of the isotopes. Metabolism can then be calculated, but only if the respiratory quotient is known or, more likely, estimated. In addition to this, the method has other limitations. Animals injected with DLW must be recaptured within about ten days before enrichment drops too low to be measured; any sooner and the error in metabolic estimation increases (Costa, 1988; Butler, 1993). The primary limitation is that the method provides only a mean estimate of metabolism over the entire period between blood samplings. Additional information on the metabolic costs of specific activities can only be elucidated if DLW is accompanied with behavioral time energy budgets (TEB) (Weathers and Nagy, 1980;

Costa et al., 1989). Speakman (1990) has also suggested that factors affecting the ratio of isotope turnover are important in the precision of the DLW technique. Diving animals (i.e., sea lions) are in continuous contact with water and could be susceptible to surface exchanges of water through the skin or respiratory surfaces. With an increase in water turnover, the isotope turnover ratio may also increase, reducing the precision of DLW. Many assumptions, controversial calculations, and other factors also compromise the validity and increase the complexity of using the technique (see Costa, 1987 and Speakman, 1993).

Heart rate recording is a technique to measure energy expenditure that offers the possibility of estimating metabolism for a year or longer with a fine time resolution of minutes or seconds that can be related to specific activities (Butler, 1993; Bevan et al., 1995b; Woakes et al., 1995; Andrews, 1998). Several comparative studies have shown that this technique is as robust as using DLW (Nolet et al., 1992; Bevan et al., 1994; Bevan et al., 1995a; Boyd et al., 1995).

The heart rate method is based on Fick's relationship relating oxygen consumption ($\dot{V}O_2$) to heart rate (f_H):

$$\dot{V}O_2 = (CaO_2 - C\bar{v}O_2) \cdot V_s \cdot f_H \quad \text{(Schmidt-Nielsen, 1990)}$$

where V_s is stroke volume of the heart, and $CaO_2 - C\bar{v}O_2$ is the difference in oxygen concentration between arterial and venous blood, respectively (also referred to as tissue oxygen extraction). Heart rate is a good indicator of $\dot{V}O_2$ if $CaO_2 - C\bar{v}O_2$ and V_s remain constant or change in proportion to changes in heart rate (Bevan et al., 1992; Butler, 1993). However, how these variables change differs among and within both terrestrial and aquatic species, and with activity (Horstman et al., 1974; Gleeson and

Baldwin, 1981; Taylor et al., 1987; Jones et al., 1989; Ponganis et al., 1990; Butler, 1993).

Several reasonably linear or curvilinear relationships have been found between f_H and $\dot{V}O_2$ when animals are exercising in a steady state. However, diving animals present a unique problem if dives are anaerobic, and single dives may not be considered a steady state given the inherent intermittency of gas exchange and large changes in heart rate that occur upon surfacing and submerging (Fedak et al., 1988; Butler, 1993). Dives, however, are rarely anaerobic (Kooyman et al., 1980; Le Boeuf et al., 1989; Thompson and Fedak, 1993), and the relationship between average heart rate and oxygen consumed may be linear over complete dive cycles (surface time plus dive) (Fedak, 1986). Significant linear relationships have been found using average dive cycles in captive diving grey seals, California sea lions, harbour seals, and bottlenose dolphins as well (Williams et al., 1991; Williams et al., 1993; Hurley, 1996).

The objective of my study was to test the feasibility of using heart rate to monitor metabolic rate in Steller sea lions using captive, juvenile animals. Alaskan Steller sea lion populations have declined to less than 30% of their peak mid 1970's abundance of about 282,000 animals (Trites and Larkin, 1996). An energy imbalance between the amount of prey consumed and the cost of obtaining it has been considered a potential reason for the decline (Alverson, 1992; Merrick et al., 1997; Rosen and Trites, 2000). However, testing this hypothesis by measuring the energetic expenditures of free-ranging Steller sea lions is difficult. Sea lions are not easily recaptured in a set time interval, and activity-specific metabolic data cannot be obtained with DLW. The heart rate method holds greater promise because data can be collected with a finer time resolution than has

so far been achieved with other methods, allowing estimation of the metabolic cost of specific activities. Long-term data can also be recorded and retrieved from dataloggers, avoiding restrictive time constraints and animal recapture.

My study tested whether a relationship exists between heart rate and oxygen consumption in each of four Steller sea lions housed at the Vancouver Aquarium Marine Science Centre. Data were collected while each animal was resting in a dry metabolic chamber or swimming, diving or resting within a swim mill. The future potential of using the heart rate technique to monitor energy expenditure of free-ranging Steller sea lions is discussed. This research sets the foundation for incorporating the heart rate method into bioenergetic studies of free-ranging Steller sea lions.

MATERIALS AND METHODS

Animals

Data were collected from four juvenile Steller sea lions, two males and two females, housed at the Vancouver Aquarium Marine Science Centre in outdoor enclosures with access to large tubs of filtered ambient seawater and haulout space. All animals followed a daily husbandry protocol consisting of scheduled feedings and training for research purposes. The study began in October 1998 when all animals were about 16 months old, and was completed in May 2000 when the animals were almost 3 years old. Over the duration of the experiment, the masses of Male 1 and 2 increased by 49.5% and 3.2%, respectively. Female 1 and 2 gained 1.1% and 3.8% in the first several days of trials then lost 3.3% and 1.0%, respectively, over the remainder of the study period (Table 1.1 and Table A1.1).

Experimental Apparatus

Monitoring heart rate: Two dorsal electrodes were attached to each sea lion in areas that gave the cleanest oscilloscope signal— either one above each scapula, or one above a scapula and the other above the pelvis on opposing sides (Fig. 1.1). Four previous styles of electrodes were tested before the final, most appropriate design, was applied (Appendix 2, Figs. A2.1-A2.4). These final electrodes consisted of two basic parts: 1) 12 cm of 28-gauge bioelectric cable inserted sub-cutaneously and 2) an external base of epoxy containing a female underwater connector soldered to the subcutaneous cable, and

Table 1.1. *Identification codes, range of mass during collection of data, and total time period of data collection for each animal.*

Animal	Animal ID	Mass Range (kg)	Time Range for Data Collection
Male 1	M97TI	102.2-152.8	October 1998 - August 1999
Male 2	M97KO	188.0-194.4	April - May 2000
Female 1	F97HA	87.4-90.4	March – September 1999
Female 2	F97SI	111.2-115.4	June – December 1999

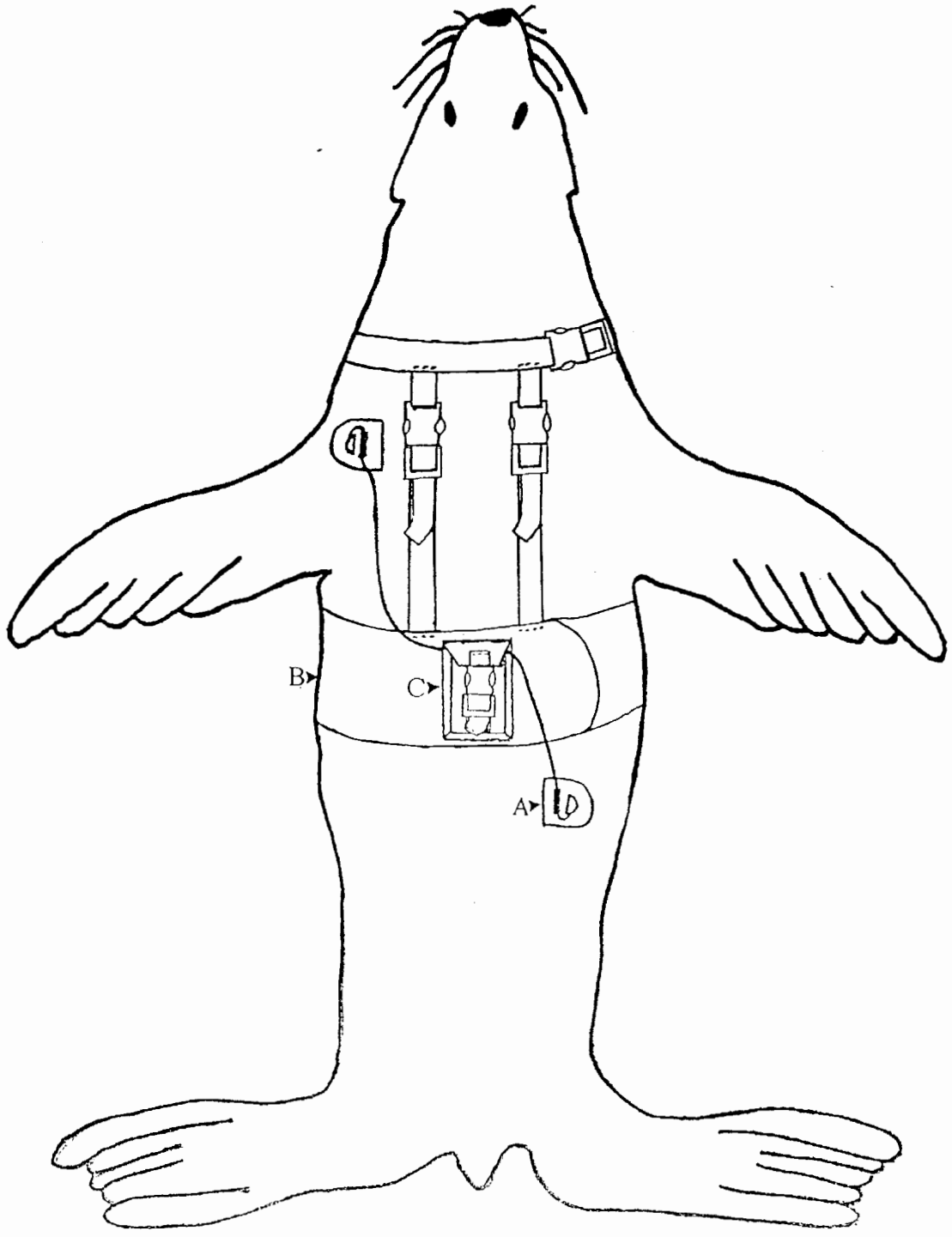

Fig. 1.1. Electrodes and harness as positioned on a captive Steller sea lion. A, electrode; B, harness; C, pocket housing the data logger.

underlain with a portion of neoprene that was glued with fast-setting superglue to the fur of the animals.

The electrocardiogram (ECG) was sampled and recorded at 100 Hz by a datalogger (R.D. Andrews, Dept. of Zoology, University of British Columbia, Vancouver, British Columbia) housed in the pocket of a nylon and neoprene harness worn by the sea lion (Brooks Wetsuits, North Vancouver, British Columbia, Fig. 1.1). The datalogger was temporarily attached to Male 2 with a spring-clip and eye system because he frequently refused to wear the harness (Appendix 3, Fig. A3.1).

Monitoring oxygen consumption: Open circuit respirometry measured oxygen consumption of each animal during dry and swimming trials as described by Rosen and Trites (1997). For dry trials, the sea lions entered a sealed opaque metabolic chamber through which air was drawn at a constant rate of 153 $L \cdot min^{-1}$. From a desiccated subsample of expired air, an S-3 A/I solid oxide cell analyzer determined oxygen concentration while an AR-60 infrared gas analyzer determined carbon dioxide concentration. A Sable Data Acquisition System calculated average concentrations from 200 subsamples of expired air every second. The system was base-lined to known ambient air concentrations before and after each trial and periodically calibrated with gases of known concentrations. The amount of oxygen consumed during a trial was calculated from the difference in oxygen concentration between airflow into and out of the chamber, with flow corrected to STPD. Air temperature within the chamber varied between 2°C and 25°C (Appendix 1, Table A1.1). A video camera and lighting within the chamber allowed for monitoring of the animal's activity.

For swimming trials, the animals entered a seawater-filled swim mill (active space= 3.2m length, 1.8m width, and 1.0m depth). The sea lions could only surface to breathe under a transparent Plexiglas dome at one end of the swim mill. Air was drawn through the dome at a rate of 141.6 L/min. The system was base-lined to known ambient air concentrations at the beginning of a day's trials and after every following hour. Oxygen and carbon dioxide concentrations were determined as above for the dry trials. To record a range of oxygen consumption and heart rates from the animals, the water current speed within the swim mill was set at 0.0, 0.9, 1.1, and 1.3 $m \cdot s^{-1}$ (0, 35, 40, and 45% maximum water load of the turbines, respectively). Male 1 was also tested at 1.5 $m \cdot s^{-1}$ (50% load)(Table A1.1). Swim speed, however, was not always equivalent to water current speed. While the current was applied, the animals often swam in a circular pattern, swimming with the current to the back of the swimmill where they would briefly rest, then swimming against the current to return to the dome to breathe. Occasionally, they would rest motionless on the bottom or beneath the dome. Water temperature varied between 2° C to 11° C for Male 1 but remained between 9° C and 11° C for the other three animals (Appendix 1, Table A1.1).

Protocol for trials

For each data-collecting period, the sea lions wore their harness with the datalogger in the pocket (or had the logger attached to clips). The free end of each of the two wires from the datalogger ended in a specialized male connector (Underwater Systems, California) to match the female connector of each electrode. Once the wires were attached to the electrodes, the logger began to record ECG. The animals were

usually fasted for the previous 12-24 hours. However, they were occasionally fed a small amount of herring (up to 200 g) prior to a session of heart rate trials to ensure co-operation. The animals were then enclosed in either the swim mill or the metabolic chamber, during which time their ECG and oxygen consumption were monitored simultaneously.

A swimming session was divided into a series of 18-minute trials. Each trial consisted of a 10-minute period to allow the animal to reach a physiological steady state at the set water current speed, to allow air to equilibrate within the enclosed system, and to make the data from each trial partially independent. Following this period, there was an 8-minute period of $\dot{V}O_2$ data collection. During each trial, the animals swam in the swimmill while one of the above water current speeds was applied. At the completion of the trial, the water current would remain the same or would be switched to a new speed and another 18-minute trial would begin. No water current was applied for the first trial in a series. For the following trials, water speed was randomly chosen using a random numbers table. If an animal became agitated swimming at a certain speed, the next trial was run without a current. A swim session continued for a maximum of 6 hours, depending on the animals' co-operation, yielding a maximum of 18 trials in a day's session. Occasionally, at the end of a session, the animals would be directed to remain as motionless as possible with their heads above water in the respirometry dome (denoted as a "hold") so that resting values in the mill could be obtained. No holds were obtained from Male 2. ECG was monitored continuously with oxygen consumption during the entire swim session.

A dry session for resting values comprised a 15-minute equilibration period once the animal was enclosed in the metabolic chamber, followed by a 15-minute $\dot{V}O_2$ data collection period. As for the swim trials, ECG was monitored continuously with oxygen consumption throughout the session. Only one dry trial was run in a day. Dry trials were obtained from only Male 1 and Female 1, as Male 2 and Female 2 would not enter the chamber.

Analysis

Heart rate: After the initial equilibration periods, mean heart rate was calculated over the following five minutes in the swim mill and the following fifteen minutes in the dry chamber. The mean heart rate during the first five minutes of a hold was also calculated. By observing ECGs from entire trials, these intervals were chosen on the basis of having stabilized heart rates or being the most representative of heart rates during a trial.

The recorded electrocardiogram was downloaded from the datalogger to a desktop personal computer after each dry or swim session using the computer communications software Crosscut Version 2.01 (Onset Computer Corp., 1994). Mean heart rate in $beats \cdot min^{-1}$ over the required interval was derived from interbeat intervals on the ECG calculated using the Acknowledge 3.0 software (Biopac Systems, Inc. and Microsoft Corp., 1992-1995)

Oxygen consumption: Mean oxygen consumption was calculated from changes in oxygen concentration and expressed in $mlO_2 \cdot min^{-1} \cdot kg^{-0.73}$ during the same time interval as heart rate described above for each trial. Metabolism has been shown to vary with body mass to a power ranging between 0.67 and 0.75 (Brody, et al. 1934; Kleiber, 1961;

Schmidt-Neilsen, 1990). I chose the exponent 0.73 as it has been generally accepted since Brody et al. (1934) first proposed it with their study on a large range of animals.

The Sable System incorporated a mean 2.8 minute delay from when air was drawn from the respirometry dome to when it was analyzed by the oxygen analyzer. To temporally synchronize oxygen consumption with heart rate, $\dot{V}O_2$ data was shifted ahead by 2.8 minutes.

***f_H/$\dot{V}O_2$* Relationships:** For each animal, a simple linear regression between f_H and the corresponding $\dot{V}O_2$ values calculated from the swim and dry trials was run using Systat (Systat, Inc.). The resulting regression was then plotted with f_H on the abscissa and $\dot{V}O_2$ on the ordinate axis. Probability levels of $p<0.05$ were considered significant. Analysis of covariance (with heart rate as the covariate) was used to compare the four resulting regressions. Residuals plotted in the order of data collection were fit with lowess-smoothed curves for each animal to detect any temporal trends in the data.

To determine a mean regression, all four data sets were pooled and fit with a mixed linear procedure (PROC MIXED, SAS) to model the means of the four animals, as well as their variances and covariances. Data from each animal were treated as a repeated measures set, as were data collected within any single day within an animal. Three possible covariance structures were considered for the repeated measurements within each animal (i.e., compound symmetry, auto regressive, and auto regressive moving average). The Akaike's Information Criteria (AIC) was used to determine the structure that gave the best fit to the pooled data.

RESULTS

Data collection

Data collection spanned 14 months between October 1998 and May 2000 (Table 1.2). Initially, data were collected infrequently (see Male 1, Table 1.2) due to the short surface electrode attachment time. With revised training and trial protocols, and improved electrode style (yielding longer attachment time), the number of trials completed daily and monthly increased.

Over the range of data collected from the four sea lions, $\dot{V}O_2$ increased between 2.3 and 4.2 times while f_H only increased between 1.6 and 2 times (Table 1.3).

Relationship between heart rate and oxygen consumption

Plotting mean heart rate (beats·min^{-1}) and the corresponding mean oxygen consumption (mlO_2·min^{-1}·kg$^{-0.73}$) from each trial showed a linear relationship between the two variables for each animal (Fig. 1.2). Slopes varied between 0.43 and 0.86 mlO_2·beat^{-1}kg$^{-0.73}$, with intercepts between 9.15 and –29.08 mlO_2·min^{-1}kg$^{-0.73}$, and r^2 values between 0.21 and 0.78 (Table 1.4).

Comparing the slopes of the four regressions (Fig 1.2) revealed that only the slopes of Female 1 and Male 2 were statistically similar. All regression intercepts were significantly different among animals (Table 1.5).

Smoothing the residuals showed that the regressions poorly described the female data collected toward the beginning and end of the study (Fig. 1.3). Possible explanations include some unknown change in equipment during collection or a physiological change in the animal (resulting in a change in the heart rate and oxygen consumption

Table 1.2. *Number of trials (holds, drys and swims) run with each of the four sub-adult Steller sea lions for each month over the entire study (October 1998 to May 2000).*

	1998			1999									2000	
Animal	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Sep	Nov	Dec	Apr	May
Male 1	2	2	2	2	6	7	2	21	5					
Male 2													27	37
Female 1						12	6			53				
Female 2								2			16	35		

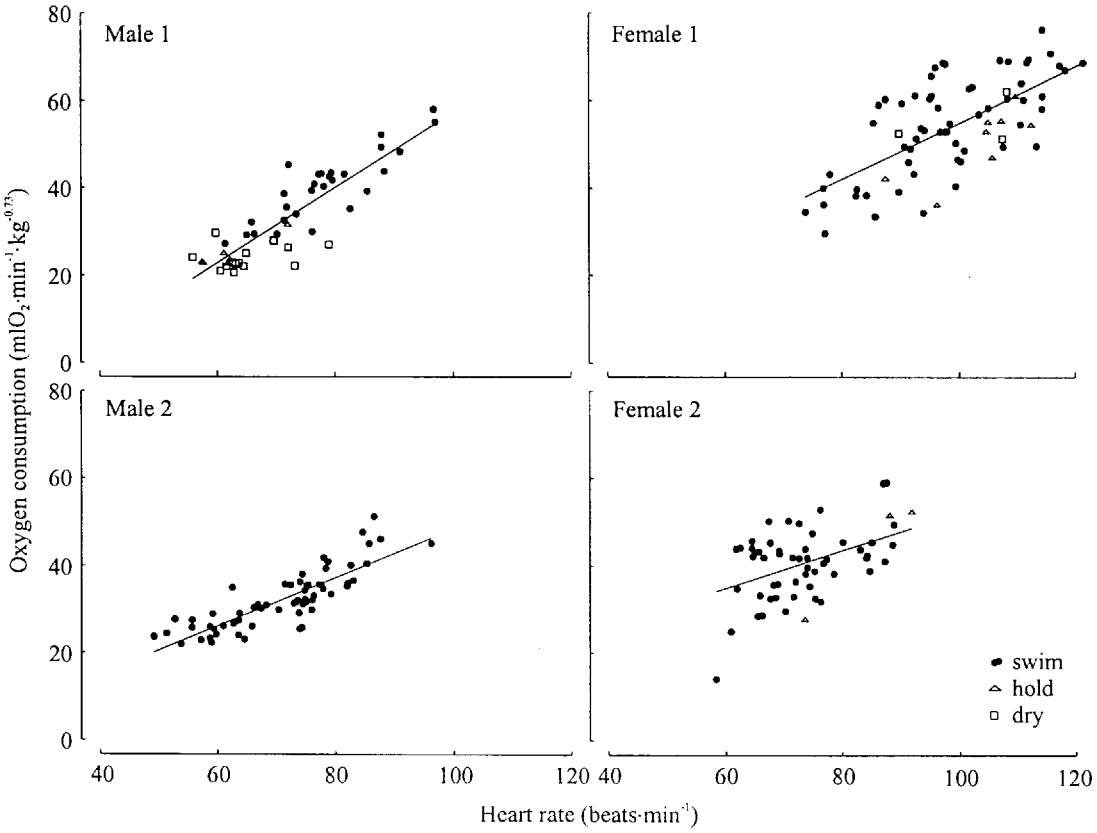

Fig. 1.2 The relationship between heart rate (beats·min^{-1}) and oxygen consumption (mlO_2·min^{-1}·kg$^{-0.73}$) in each of the four sub-adult Steller sea lions, plotted with the corresponding least-squares fitted linear regression. Each regression was fit to all types of data available in an animal's set: swim and dry trials, and holds.

Table 1.3. *Magnitude of increase in* $\dot{V}O_2$ *and* f_H *over the range of data recorded in all four Steller sea lions, and the ratio of the increases.*

Animal	Magnitude of Increase in $\dot{V}O_2$	Magnitude of Increase in f_H	$f_H/\dot{V}O_2$
Male 1	2.8	1.7	0.61
Male 2	2.3	2	0.87
Female 1	2.6	1.7	0.65
Female 2	4.2	1.6	0.38

Table 1.4. *Slopes, intercepts, and r^2 values of the four individual Steller sea lion regression lines shown in Fig 1.2.*

Animal	Slope (±SE)	Intercept (±SE)	n	r^2
Male 1	0.86 ± 0.07	-29.08 ± 4.84	49	0.78
Male 2	0.56 ± 0.05	-7.43 ± 3.27	64	0.71
Female 1	0.64 ± 0.08	-9.23 ± 8.50	71	0.45
Female 2	0.43 ± 0.12	9.15 ± 8.68	53	0.21

Table 1.5. *P-values from slope and elevation comparisons of all four linear sea lion regressions. Significant differences (at the p<0.05 level) are in bold.*

	Male 1	Male 2	Female 1
Slopes			
Male 2	**<0.001**		
Female 1	**<0.001**	0.14	
Female 2	**=0.001**	**=0.005**	**<0.001**
Elevations			
Male 2	**<0.001**		
Female 1	**<0.001**	**<0.001**	
Female 2	**<0.001**	**<0.001**	**<0.001**

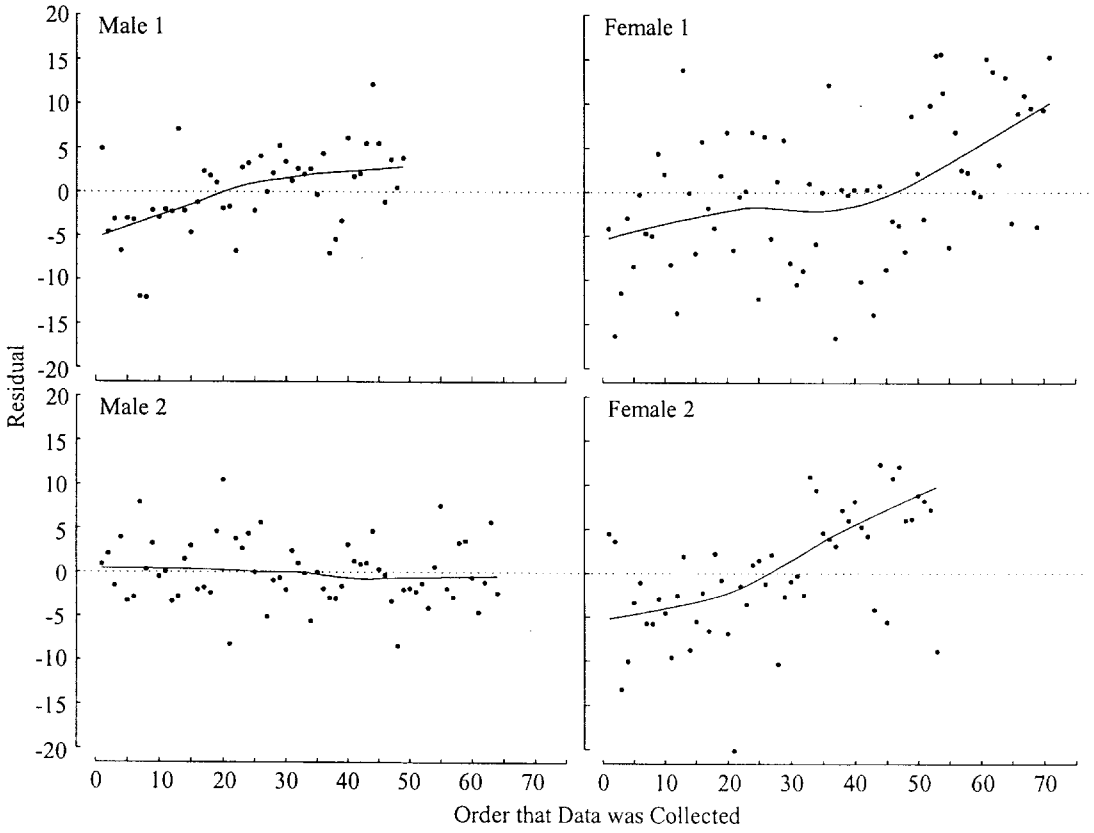

Fig. 1.3. Residuals (y-axis) in chronological order of data collection (x-axis) for each of the four sub-adult Steller sea lions. Lowess-smoothed curves have been fit to the data.

relationship). A similar but slighter trend is evident for Male 1, while residuals from Male 2 were evenly distributed about a mean of zero (Fig. 1.3).

A mean regression for all four Steller sea lions could be determined by calculating the mean slope and intercept of the four linear regressions (Table 1.3, Fig 1.4a). However, a better method is to pool the four data sets and fit the resulting set with a mixed linear model, treating all data from an animal, and from a day within an animal, as a repeated measures set. Out of the three covariance structures examined to consider the repeated measures, the compound symmetrical structure yielded the lowest Akaike's Information Criteria (1518.8 compared with 1519.0 for the autoregressive and 1547.3 for the autoregressive moving average structures), and therefore gave the best fitting linear model. The mean regression resulting from this analysis was

$\dot{V}O_2 = (0.68 f_H \pm 0.07) - (15.07 \pm 6.20)$ ($r^2 = 0.72$, $p < 0.01$) and is shown in Fig. 1.4b.

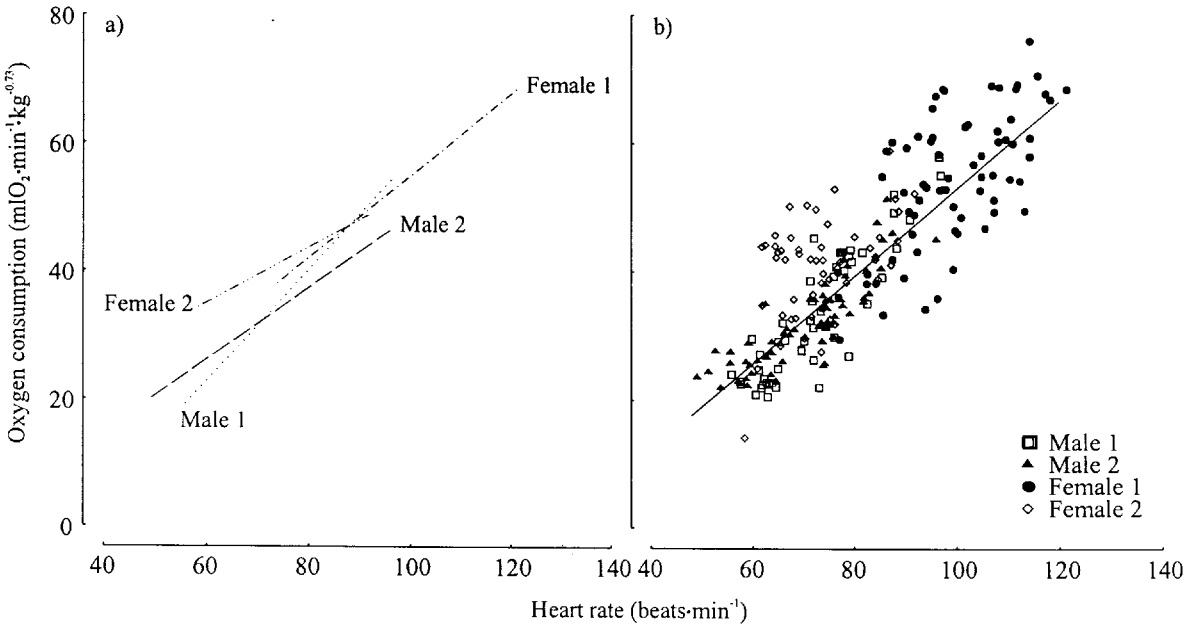

Fig. 1.4. a) Linear regressions (from Fig. 1.2) for all four sea lions and b) the mean regression ($\dot{V}O_2$= (0.68fH ± 0.07) - (15.07 ± 6.20), r^2=0.72) shown with all sea lion data.

DISCUSSION

As shown for a number of species, the relationship between heart rate and metabolism in fasted Steller sea lions is linear. However, there was considerable variability among some of the data collected for the two females and the regressions for the four individuals were not all statistically the same. It remains unclear whether the mean regression I calculated for the four animals can be applied to wild individuals.

Factors Affecting the $f_H/\dot{V}O_2$ Relationship

A number of factors may explain the variability in the measurements of heart rate and oxygen consumption within and between individual animals (Fig. 1.2). They include possible changes or differences in stress level, fitness, and other physiological parameters of the study animals.

Considerable data on humans, dogs, and rhesus monkeys have shown that psychological stress brought on by threatening stimuli can lead to heart rate variations that do not always correspond positively with variations in metabolism (Johnson and Gessaman, 1973; Obrist et al., 1974; Stromme et al., 1978; Langer et al., 1979; Langer et al., 1985). Stress (through the release of epinephrine to beta-adrenoreceptors) can cause the heart rate to increase beyond that which can be predicted from measured oxygen uptake (using $f_H/\dot{V}O_2$ relationships derived from treadmill exercise without psychological stress) (Blix et al., 1974; Stromme et al., 1978; Turner and Carroll, 1985; Wilhelm and Roth, 1998).

Physical fitness, which increases the functional capacity of the cardiovascular system, is a second factor that may potentially affect the $f_H/\dot{V}O_2$ relationship. As fitness improves, the heart enlarges, ventricular stretching is enhanced, and blood volume

increases, resulting in an increased stroke volume allowing reduction in the heart rate for a given oxygen consumption (Plowman and Smith, 1997). These changes raise the intercept of the $f_H/\dot{V}O_2$ relationship relative to that of more sedentary individuals (Swaine et al., 1992; Mtinangi and Hainsworth, 1999).

A third factor to consider is potential ontogenic changes including mass fluctuations. However, unfortunately, papers specifically discussing the effects of ontogenic factors on the $f_H/\dot{V}O_2$ relationship were not found.

In general, growth and development result in an increase in mass after birth. With an increase in body size, the mass of the heart will also increase in a nearly proportionate manner (heart mass = $0.006M_b^{0.98}$, where M_b is body mass). Stroke volume also increases with body mass. Heart rate, however, is generally inversely related to body mass ($f_H = 241M_b^{-0.25}$) (Schmidt-Nielsen, 1990; Farrell, 1991). If $\dot{V}O_2$ does not vary with mass in a similar manner as heart rate above, the $f_H/\dot{V}O_2$ relationship would change with any mass variations of the animal. If this were true, applying the heart rate method (without considering mass changes) to growing or fasting animals would lead to inaccurate metabolic estimations.

An increase in body fat will result in an increase in body mass— however, a change in metabolism may not be experienced. Cunningham (1991) has shown that fat-free body mass is the primary predictor of resting metabolic rate while fat mass is insignificant. However, Ferraro and Ravussi (1992) stated that fat mass is significant in determining metabolism. If metabolism does not change with an increase in body fat, the $f_H/\dot{V}O_2$ relationship would be altered. If metabolism does change, it must change in an equal proportion with heart rate for the $f_H/\dot{V}O_2$ relationship to remain unaltered.

It is not clear to what extent the above factors may have affected the $f_H/\dot{V}O_2$ relationship of my study animals. The negative to positive trend in the regression residuals (plotted in the order of data collection) of the female Steller sea lions showed initially high heart rates for a given oxygen consumption and suggest that stress may have decreased and/or that fitness increased in these animals as my study progressed (Fig. 1.3). Body mass changes were insignificant and were not considered responsible for the trends. However, total body mass may have remained the same but the body composition of fat may have changed. This may have been a factor resulting in the trend in the residuals. A high r^2 value and slight trend in the regression residuals of Male 1 suggests a possible effect of stress, fitness or a change in body fat composition over time on f_H and $\dot{V}O_2$, but relatively little compared to the females, despite the longer data collection period. This animal experienced a 49.5% mass increase over the study that may also have had some influence on the negative to positive trend observed. The lack of a trend and high r^2 in Male 2 suggests no effect of stress, fitness, changing body composition, or mass changes (which were insignificant as in the females) over time.

Stroke Volume and Oxygen Extraction

The validity of the heart rate method lies with the changes in stroke volume and arterio-venous oxygen extraction. These parameters must remain constant or vary in a predictable, systematic manner for heart rate to reliably estimate $\dot{V}O_2$.

The r^2 values in Table 1.4 suggest that f_H explains between 71 and 78% of the variation in measured $\dot{V}O_2$ for the males and between 21 and 45% of the increase in the females. However, looking closely at the data in Fig 1.2, $\dot{V}O_2$ increased about 2.5 to 4

times while f_H only doubled across the ranges recorded in the four sea lions (Table 1.3). This illustrates that the heart rate data can physiologically explain only between 38% and 87% of the increase in $\dot{V}O_2$. A systematically increasing stroke volume and/or tissue oxygen extraction must therefore explain the remaining increase in $\dot{V}O_2$ if heart rate is to be truly linearly related to $\dot{V}O_2$ — which may or may not be true in Steller sea lions.

Ideally, stroke volume and tissue oxygen extraction should be measured as they have been in other mammals. In horses, steers, goats, calves, and some dogs, mass-specific stroke volume remains constant over a range of exercise (Horstman et al., 1974; Taylor et al., 1987; Jones et al., 1989). Oxygen extraction, however, increased with exercise in a systematic curvilinear manner. In rats and some dogs, both stroke volume and extraction increased in a linear fashion with exercise (Gleeson and Baldwin, 1981; Horstman et al., 1974). Similar changes in Steller sea lions (i.e., a systematically increasing stroke volume and/or extraction) would help explain the remainder of the increase in $\dot{V}O_2$, and the f_H/ $\dot{V}O_2$ relationship would be linear.

The changes in stroke volume and oxygen extraction are more complex in marine mammals because the oxygen demands of swimming conflict with the need to conserve oxygen while diving, leading to complex variation in these physiological parameters. Over a range of workloads, harbour seals show a slightly decreasing surface-swimming stroke volume that is twice the constant level recorded while swimming submerged (Ponganis et al., 1990); oxygen extraction was not reported. California sea lions, on the other hand, have been shown to maintain a constant stroke volume over a continuous period of short submergence and surface swimming events, regardless of workload (Ponganis et al., 1991). Data was not separated into surface and dive intervals. Again,

extraction was not reported. Stroke volume changes in Steller sea lions are probably similar to California sea lions, since swimming in my study involved short dive and surface events. If this is true, and stroke volume remains constant, extraction must be responsible for the increase in $\dot{V}O_2$ that is not explained by f_H. However, without recorded values, what is occurring cannot be explained conclusively.

Steller Sea Lion Regressions and Data Collection

A considerable amount of effort was put into trying to increase the range of heart rates and oxygen consumption used in my regressions. One approach was to try to increase swimming speeds by setting faster water currents in the swimmill. Unfortunately, some animals would not swim when the current was set beyond 1.11 $m \cdot s^{-1}$. Another approach was to insert 5.4 kg of weights into the harness worn by Male 1, but this too failed to increase oxygen consumption beyond that monitored while swimming without the harness. A third alternative was to feed an animal a bulk amount of food preceding a trial. This drastically affected the slope of the regression, and produced data that could not be used to establish the baseline $f_H/\dot{V}O_2$ relationship (Appendix 4).

A certain amount of variability in data collection is normal and does not mean that the heart rate method is inadequate, especially relative to other available techniques. It may have been possible to reduce some of the variation in the Steller sea lion data set by experimentally collecting data within 24 hours. This has been done previously in some captive marine bird and mammal studies where significant $f_H/\dot{V}O_2$ relationships were obtained with minimal variation (r^2 values were above 0.70) (see Butler et al., 1992; Bevan et al., 1994; Williams et al., 1991; Bevan et al., 1995). Prompt data collection on

captive animals would likely reduce variation in development, fitness levels, and stress. However, the application of the regressions to wild Steller sea lions living in the open ocean will likely be done over several days or months. Thus, it is likely more meaningful to calibrate regressions on captive animals over an extended time period, as I have done, rather than in a single day as some others have done (i.e., Butler et al., 1992; Bevan et al., 1994; Bevan et al., 1995; Williams et al., 1991).

Wild Steller sea lions would continuously be exposed to stressful stimuli and events (i.e., boats, predators, locating and capturing prey) and would experience changes in development, fitness, and changes in physiological parameters throughout a monitoring session. Because of this, determining and applying a mean regression over a long period may be superior to applying one determined over a very narrow time interval that does not encompass natural factors of variation. Variation about a mean regression may increase because of this, but its application may be more meaningful.

Comparison with Other Methods and Species

Studies on other species such as California sea lions (Butler et al., 1992; Boyd et al., 1995), barnacle geese (Nolet et al., 1992), gentoo penguins (Bevan et al., 1995), and black-browed albatrosses (Bevan et al., 1994) have concluded that heart rate is a good monitor of metabolism despite significant differences between slopes and intercepts of individual regressions. These studies compared predicted to observed oxygen consumption and found that heart rate could predict oxygen consumption with an error less than or equivalent to that predicted by doubly-labelled water. In captive swimming California sea lions, DLW overestimated metabolic rate by as much as 36.4% on average

(range –10% to 86%), while the range of error using heart rate was only from –28% to +23% (mean 2.7%) (Boyd et al., 1995). However, both techniques were considered valid. Thus, although regressions in my study differed among individuals (Table 1.5), heart rate may still yield a better estimate of oxygen consumption relative to the doubly-labelled water technique. However, validation experiments to determine the mean regression's ability to closely estimate metabolism are still needed before it can be applied with confidence to estimate the mean metabolism of groups of Steller sea lions in the field.

Nagy (1989) stated that the accuracy of DLW technique is commonly about ±4% in captive mammals and birds, increasing to ±8 in free-living animals. The large error mentioned above for California sea lions most likely lies in the short 24-hour experimental period and is, thus, another shortcoming of the DLW method. The accuracy of time-energy budgeting in estimating DLW metabolism measurements can range between -44% and +57%— however, most of this research was performed on birds (Nagy, 1989). With the estimated ±8% error in DLW measurements, time energy budgets may underestimate actual metabolism by 52%, or overestimate it by 65%. Unfortunately, marine mammal studies reporting error in metabolism estimates using time energy budgets were not found. However, error may be very large considering a diving animal's activity cannot be observed at all times. Time energy budgets also encompass a possible error in assuming metabolic measurements from a captive animal would be similar to metabolic measurements in a wild animal engaged in a similar activity. These errors inherent with DLW and TEB improve the suitability of the heart rate method.

The resulting mean $f_H/\dot{V}O_2$ regression, $\dot{V}O_2 = (0.68\, f_H \pm 0.07) - (15.07 \pm 6.20)$, determined from the four Steller sea lions in my study had a reasonably tight fit to the data (r^2=0.72) (Fig. 1.4a). However, for comparison with other marine mammal studies, the mixed linear models were re-run to determine a mean regression with oxygen consumption measured in ml $O_2 \cdot min^{-1} \cdot kg^{-1}$. The resulting regression, $\dot{V}O_2 = (0.19 f_H \pm 0.02) - (4.22 \pm 1.68)$, is different from that determined in the other marine mammal species (Fig. 1.5). Regressions of f_H and $\dot{V}O_2$ appear to be species-specific, ruling out the notion of applying one overall regression for use in estimating heart rate in all marine mammal species or adapting a regression from one species to another. As expected from their larger body mass and illustrated in Fig. 1.4a, males generally consumed less oxygen per kilogram$^{0.73}$ of mass than the females, indicating that there may even be separate regressions for the sexes, an idea suggested by Hurley (1996) with California sea lions that should be explored further.

Captivity versus Free-ranging Environment

The assumption that a $f_H/\dot{V}O_2$ relationship determined from captive animals is similar to that existing in free-ranging animals warrants discussion. Accurately calibrating a relationship in free-ranging animals undergoing completely natural, undisturbed behaviour is currently difficult, if not impossible. Direct respirometry cannot be performed in the wild and applying heart rate to monitor metabolism using a regression between heart rate and oxygen consumption estimated with DLW and/or TEB may result in large errors in true metabolism. I do not know to what extent my results were affected by the confining nature of the swimmill and metabolic chamber. An

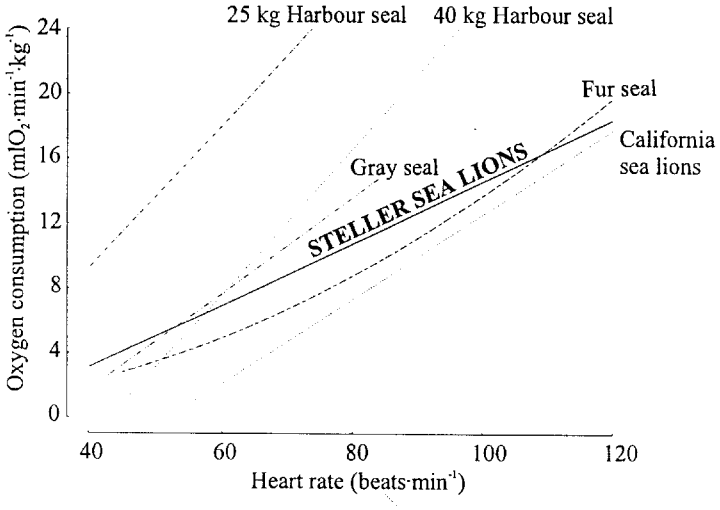

Fig. 1.5. The relationship between heart rate (beats·min^{-1}) and oxygen consumption (mlO_2·min^{-1}·kg^{-1}) in Steller sea lions ($\dot{V}O_2$= 0.19fH ± 0.02) - (4.22 ± 1.68), r^2=0.69) shown, for comparison, with that determined in various other pinniped species.

obvious next step would be to collect similar data from the animals swimming in a much larger pool equipped for direct respirometry, or swimming beneath a respirometry hood alongside a boat in the open ocean. Both approaches offer reduced confinement and may yield more natural physiology. Regressions determined from such data could then be used to estimate metabolism from heart rate in free-ranging animals with more confidence. In the interim, however, the regressions I have calculated can be applied to free-ranging Steller sea lions (with the obvious caveats) and should be updated as further experimentation with this technique continues.

Future Research

From the data presented here, it appears that a good linear relationship between heart rate and oxygen consumption exists in young male captive Steller sea lions swimming in a swim mill with a varying water current. This indicates that heart rate may reliably estimate metabolism in male Steller sea lions. In female Steller sea lions, however, the relationship appears linear but with much more variation. This suggests that heart rate may not be as reliable for estimating metabolism in females. Factors like changes in stress and fitness levels may have a greater affect on the $f_H/\dot{V}O_2$ relationship in females, or stroke volume and tissue oxygen extraction may not be changing as systematically as they appear to in males.

The reasonably tight fit of the mean regression (Fig. 1.4b) indicates that heart rate varies linearly with oxygen consumption, thus supporting the idea that heart rate can reliably estimate metabolism in all Steller sea lions when the mean regression is used. This is in accordance with previous terrestrial and aquatic animal studies that have

reported that mean regressions can accurately estimate metabolism in individuals (Butler, 1993). However, because it is unknown whether a fixed relationship exists between heart rate and oxygen consumption under all conditions encountered, more detailed examination of the regressions should be undertaken with experimental conditions that would occur naturally in the wild (i.e., food, foraging, stress, changing body compositions, development, etc.). Varying food intake, introducing live fish to simulate hunting, inducing varying stress levels are all examples of simulated field conditions that should be considered to investigate how closely a regression estimates metabolism under such situations.

SUMMARY

The potential use of heart rate to monitor energy expenditure in free-ranging Steller sea lions was explored by establishing whether a relationship exists between heart rate (f_H) and oxygen consumption ($\dot{V}O_2$) in resting and swimming sea lions.

ECG and $\dot{V}O_2$ were recorded simultaneously from four fasted juvenile sea lions while resting in a dry metabolic chamber or while swimming in a swim mill with various water current speeds applied (up to 1.5 $m \cdot s^{-1}$). Mean $\dot{V}O_2$ was calculated for each trial (a mean of 59 trials per animal) and regressed against mean f_H for each animal. The relationship between $\dot{V}O_2$ and f_H was linear over the range of heart rates that were recorded. However, slopes and intercepts differed between some of the animals, possibly due to differences in fitness, stress, and/or changes in stroke volume and tissue oxygen extraction. The mean regression equation for all four animals was $\dot{V}O_2 = (0.68 f_H \pm 0.07\text{s.e.}) - (15.07 \pm 6.20)$ ($r^2=0.72$, $p<0.01$).

The relationship between f_H and $\dot{V}O_2$ suggests that heart rate can potentially be used to monitor energy consumption in free-ranging Steller sea lions. However, the relationship should be further investigated to determine how it is affected by such factors as feeding, sex, stress, and development.

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Appendix 1: Complete Fasting Data Set

Table A1.1. Complete fasting data set collected from the four juvenile Steller sea lions.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Male 1	16-Oct-98	dry	0.0	42301.75	24.06	55.82	102.2	N/A	09.18.16	0.00
	30-Oct-98	dry	0.0	39336.50	22.06	64.52	104.2	N/A	10.52.13	0.00
	4-Nov-98	dry	0.0	40781.30	22.81	63.70	104.6	N/A	11.03.16	0.00
	12-Nov-98	dry	0.0	48087.40	26.38	72.01	107.4	11.5	10.07.09	0.00
	17-Dec-98	dry	0.0	52472.40	27.95	69.55	111.8	11.9	09.45.08	0.00
	22-Dec-98	dry	0.0	51823.70	27.79	69.57	110.8	3.4	10.49.05	0.00
	8-Jan-99	dry	0.0	44409.50	22.12	73.16	122.6	6.0	10.51.12	0.00
	12-Jan-99	dry	0.0	54516.83	27.02	78.95	123.4	7.7	09.59.07	0.00
	18-Feb-99	dry	0.0	44935.90	21.99	61.60	125.6	5.6	09.58.54	0.00
	20-Feb-99	dry	0.0	46712.30	22.57	63.17	127.8	4.8	10.07.22	0.00
	22-Feb-99	dry	0.0	51933.60	24.98	64.92	128.6	5.4	10.03.24	0.00
	23-Feb-99	dry	0.0	43566.00	20.95	60.55	128.6	4.3	10.55.03	0.00
	25-Feb-99	hold	0.0	61990.73	29.65	59.77	129.6	6.0	09.39.46	0.00
	25-Feb-99	swim	0.0	47804.45	22.86	62.66	129.6	6.0	09.39.46	0.00
	11-Mar-99	swim	0.0	42845.82	20.58	62.86	128.8	8.5	10.02.39	0.00
	11-Mar-99	hold	0.0	48818.70	23.45	62.21	128.8	8.5	10.21.00	0.00
	22-Mar-99	hold	0.0	48875.34	22.96	57.49	132.8	7.0	10.10.10	0.00
	23-Mar-99	dry	0.0	48528.22	22.58	57.63	134.6	9.3	10.29.21	0.00
	27-Mar-99	dry	0.0	53958.08	24.83	61.17	136.6	8.0	11.44.57	0.00
	29-Mar-99	dry	0.0	48622.99	22.50	61.90	135.6	6.1	12.43.18	0.00
	30-Mar-99	hold	0.0	68097.51	31.37	71.97	136.4	2.0	10.17.58	0.00
	1-Apr-99	hold	0.0	64925.67	29.93	76.14	136.3	3.5	09.58.32	0.00
	1-Apr-99	hold	0.0	85446.11	39.39	76.06	136.3	3.5	10.41.24	0.00
	1-Jun-99	swim	0.0	61864.96	27.20	61.38	145.4	11.5	09.43.49	0.00
	1-Jun-99	swim	0.9	66695.49	29.33	70.11	145.4	10.5	10.03.24	0.00
	1-Jun-99	swim	1.5	98908.51	43.49	79.34	145.4	10.0	10.23.10	0.00
	1-Jun-99	swim	0.0	74027.05	32.55	71.36	145.4	10.0	10.42.22	0.00

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Male 1	1-Jun-99	swim	0.0	66384.17	29.19	65.00	145.4	10.5	10.59.23	0.00
	1-Jun-99	swim	1.5	98371.12	43.26	77.70	145.4	10.5	11.27.28	0.00
	1-Jun-99	swim	1.3	96930.75	42.62	79.04	145.4	10.5	11.48.45	0.00
	1-Jun-99	swim	0.9	66989.71	29.46	66.30	145.4	10.5	12.08.27	0.00
	11-Jun-99	swim	0.0	82278.50	35.57	71.78	148.8	9.0	11.36.02	0.00
	11-Jun-99	swim	1.1	93359.50	40.37	78.10	148.8	9.5	11.55.18	0.00
	11-Jun-99	swim	1.3	114155.60	49.36	87.78	148.8	10.0	12.14.31	0.00
	11-Jun-99	swim	1.5	78645.41	34.00	73.41	148.8	10.0	12.34.03	0.00
	11-Jun-99	swim	0.0	74319.16	32.13	65.86	148.8	10.5	12.53.07	0.00
	11-Jun-99	swim	0.9	81397.52	35.19	82.54	148.8	10.5	13.27.04	0.00
	11-Jun-99	swim	1.3	90773.21	39.25	85.40	148.8	10.5	13.48.18	0.00
	11-Jun-99	swim	1.5	101309.60	43.80	88.26	148.8	10.5	14.09.33	0.00
	11-Jun-99	swim	1.1	89429.16	38.67	71.39	148.8	10.0	14.30.48	0.00
	11-Jun-99	swim	0.9	99810.77	43.15	81.61	148.8	10.0	14.52.05	0.00
	11-Jun-99	swim	1.1	96472.63	41.71	79.59	148.8	10.0	15.13.01	0.00
	11-Jun-99	swim	1.5	99842.23	43.17	77.31	148.8	10.0	15.33.30	0.00
	11-Jun-99	swim	1.3	104854.80	45.34	72.12	148.8	10.0	15.53.31	0.00
	21-Aug-99	swim	0.0	123101.10	52.20	87.78	152.8	10.0	10.50.39	0.10
	21-Aug-99	swim	0.9	113856.10	48.28	90.92	152.8	10.0	11.10.44	0.10
	21-Aug-99	swim	1.1	136911.80	58.06	96.67	152.8	10.5	11.29.39	0.10
	21-Aug-99	swim	1.3	130008.70	55.13	96.97	152.8	10.5	11.48.43	0.10
	21-Aug-99	swim	0.0	96352.50	40.86	76.53	152.8	10.5	12.07.40	0.10
Male 2	28-Apr-00	swim	0.0	79839.02	29.10	63.69	188.0	10.0	14:26:00	0.50
	28-Apr-00	swim	0.0	70695.76	25.77	55.58	188.0	10.0	14:43:12	0.50
	28-Apr-00	swim	0.9	88178.46	32.14	73.51	188.0	10.0	15:02:08	0.50
	28-Apr-00	swim	0.9	104489.90	38.09	74.35	188.0	10.0	15:23:03	0.50
	28-Apr-00	swim	0.0	61064.99	22.26	58.91	188.0	10.0	16:19:57	0.50
	28-Apr-00	swim	0.9	86132.09	31.40	74.58	188.0	10.0	16:38:26	0.50
	28-Apr-00	swim	1.1	130948.30	47.73	84.47	188	10.0	16:57:19	0.50

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Male 2	28-Apr-00	swim	0.0	85079.81	31.01	68.15	188.0	10.0	17:14:57	0.50
	29-Apr-00	swim	0.0	79684.10	28.91	59.11	189.2	10.0	10:13:21	0.50
	29-Apr-00	swim	0.0	72046.40	26.14	60.92	189.2	10.0	10:31:11	0.50
	29-Apr-00	swim	0.9	94791.04	34.39	74.70	189.2	10.0	10:49:49	0.50
	29-Apr-00	swim	0.9	92389.80	33.52	79.20	189.2	10.0	11:07:54	0.50
	29-Apr-00	swim	0.9	87371.84	31.70	74.99	189.2	10.0	11:26:30	0.50
	29-Apr-00	swim	1.1	110712.00	40.17	82.48	189.2	10.0	11:45:18	0.50
	29-Apr-00	swim	1.1	108577.40	39.39	78.35	189.2	10.0	12:03:57	0.50
	29-Apr-00	swim	0.0	64312.20	23.33	58.64	189.2	10.0	12:22:25	0.50
	29-Apr-00	swim	0.0	66622.29	24.17	59.71	189.2	10.0	12:41:03	0.50
	29-Apr-00	swim	0.9	87832.50	31.87	74.58	189.2	10.0	13:00:09	0.50
	29-Apr-00	swim	1.1	127046.70	46.10	87.47	189.2	10.0	13:19:11	0.50
	29-Apr-00	swim	1.1	141535.10	51.35	86.38	189.2	10.0	13:37:30	0.50
	29-Apr-00	swim	0.0	71112.18	25.80	74.23	189.2	10.0	13:56:25	0.50
	29-Apr-00	swim	0.0	75758.20	27.49	55.63	189.2	10.0	14:15:08	0.50
	29-Apr-00	swim	0.9	98430.66	35.71	72.31	189.2	10.0	14:34:24	0.50
	29-Apr-00	swim	1.1	112826.70	40.94	78.67	189.2	10.0	14:53:20	0.50
	29-Apr-00	swim	1.1	98469.72	35.73	77.18	189.2	10.0	15:12:40	0.50
	29-Apr-00	swim	0.0	76206.30	27.65	52.70	189.2	10.0	15:31:26	0.50
	29-Apr-00	swim	0.0	82439.48	29.91	75.94	189.2	10.0	15:49:47	0.50
	2-May-00	swim	0.0	73800.20	26.69	62.72	190.0	10.0	09.35.32	0.40
	2-May-00	swim	0.0	75911.71	27.46	63.59	190.0	10.0	09.53.45	0.40
	2-May-00	swim	0.9	91643.44	33.15	76.24	190.0	10.0	10:12:48	0.40
	2-May-00	swim	0.9	100488.90	36.35	73.93	190.0	10.0	10:31:26	0.40
	2-May-00	swim	1.1	98748.49	35.72	75.34	190.0	10.0	10:51:21	0.40
	2-May-00	swim	1.1	98752.83	35.72	77.42	190.0	10.0	11:09:47	0.40
	2-May-00	swim	0.0	63694.38	23.04	64.53	190.0	10.0	11:28:30	0.40
	2-May-00	swim	0.9	83467.89	30.19	67.29	190.0	10.0	11:48:02	0.40
	2-May-00	swim	0.9	82747.23	29.93	70.27	190.0	10.0	12:06:16	0.40

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Male 2	2-May-00	swim	1.1	97777.45	35.37	81.85	190.0	10.0	12:25:01	0.40
	2-May-00	swim	1.1	86139.02	31.16	74.40	190.0	10.0	12:42:37	0.40
	2-May-00	swim	0.0	63296.03	22.89	57.12	190.0	10.0	13:01:36	0.40
	2-May-00	swim	0.0	67550.84	24.43	51.33	190.0	10.0	13:19:46	0.40
	2-May-00	swim	0.9	86024.19	31.12	66.73	190.0	10.0	13:38:55	0.40
	2-May-00	swim	0.9	84223.45	30.46	66.12	190.0	10.0	13:57:17	0.40
	2-May-00	swim	1.1	98416.89	35.60	75.12	190.0	10.0	14:16:28	0.40
	2-May-00	swim	1.3	124650.10	45.09	85.57	190.0	10.0	14:39:05	0.40
	2-May-00	swim	1.3	112073.30	40.54	85.21	190.0	10.0	14:57:21	0.40
	2-May-00	swim	0.0	70342.59	25.44	59.38	190.0	10.0	15:16:14	0.30
	3-May-00	swim	0.0	73228.09	26.05	65.80	194.4	10.0	09.09.03	0.30
	3-May-00	swim	0.0	71576.07	25.46	73.91	194.4	10.0	09.27.19	0.30
	3-May-00	swim	0.9	91000.95	32.37	74.80	194.4	10.0	09.46.46	0.30
	3-May-00	swim	0.9	88488.57	31.48	72.92	194.4	10.0	10:05:13	0.30
	3-May-00	swim	1.1	103099.50	36.67	82.89	194.4	10.0	10:24:31	0.30
	3-May-00	swim	1.1	97734.70	34.77	77.81	194.4	10.0	10:43:05	0.30
	3-May-00	swim	0.0	67446.59	23.99	63.53	194.4	10.0	11:01:36	0.30
	3-May-00	swim	0.0	73049.64	25.98	58.65	194.4	10.0	11:19:54	0.30
	3-May-00	swim	0.9	98472.36	35.03	62.49	194.4	10.0	11:38:50	0.30
	3-May-00	swim	0.9	88716.55	31.56	73.15	194.4	10.0	11:57:29	0.30
	3-May-00	swim	1.1	90452.07	32.18	76.02	194.4	10.0	12:16:44	0.30
	3-May-00	swim	1.1	100682.50	35.81	71.37	194.4	10.0	12:35:59	0.30
	3-May-00	swim	0.0	66369.80	23.61	49.14	194.4	10.0	12:54:57	0.30
	3-May-00	swim	0.0	61754.90	21.97	53.73	194.4	10.0	13:13:55	0.30
	3-May-00	swim	0.9	82083.43	29.20	73.74	194.4	10.0	13:33:00	0.30
	3-May-00	swim	1.1	126843.50	45.12	96.07	194.4	10.0	13:52:05	0.30
	3-May-00	swim	1.3	117657.00	41.85	77.99	194.4	10.0	14:11:35	0.30
	3-May-00	swim	1.3	101219.90	36.01	81.98	194.4	10.0	14:30:29	0.30
Female 1	22-Mar-99	swim	0.0	88034.60	55.21	107.32	89.4	7.5	10.46.42	0.00

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Female 1	22-Mar-99	hold	N/A	57429.11	36.02	96.29	89.4	7.5	11.07.30	0.00
	27-Mar-99	swim	0.0	75363.34	46.88	105.73	90.4	4.5	10.05.36	0.00
	27-Mar-99	hold	N/A	88310.48	54.94	105.03	90.4	4.5	10.23.58	0.00
	29-Mar-99	swim	0.0	87198.59	54.24	112.53	90.4	7.5	10.45.54	0.00
	29-Mar-99	hold	N/A	97602.50	60.72	109.82	90.4	7.5	11.11.06	0.00
	31-Mar-99	swim	0.0	66953.44	41.99	87.46	89.4	2.0	11.00.57	0.00
	31-Mar-99	hold	N/A	84123.09	52.76	104.74	89.4	2.0	11.22.18	0.00
	1-Apr-99	swim	0.0	83184.63	52.51	89.71	88.6	4.5	12.03.33	0.00
	1-Apr-99	hold	N/A	98244.80	62.02	108.24	88.6	4.5	12.19.32	0.00
	6-Apr-99	swim	0.0	81207.80	51.26	107.49	88.6	7.5	09.43.49	0.00
	6-Apr-99	hold	N/A	78533.76	49.58	113.45	88.6	7.5	10.01.07	0.00
	7-Apr-99	dry	N/A	103864.20	65.57	95.37	88.6	7.5	12.57.55	0.00
	8-Apr-99	swim	0.0	90069.00	56.86	103.48	88.6	4.5	10.08.13	0.00
	8-Apr-99	hold	N/A	86405.00	54.55	110.65	88.6	4.5	10.24.14	0.00
	17-Sep-99	swim	0.0	110774.20	70.63	115.94	87.4	11.5	09.39.29	0.50
	17-Sep-99	swim	0.9	94245.70	60.09	111.20	87.4	10.5	09.58.15	0.50
	18-Sep-99	swim	0.0	78873.60	50.29	99.50	87.4	9.5	08.53.08	0.00
	18-Sep-99	swim	0.9	106375.40	67.82	117.49	87.4	9.0	09.12.22	0.00
	18-Sep-99	swim	1.1	108612.10	69.25	112.13	87.4	9.5	09.30.54	0.00
	18-Sep-99	swim	0.0	67822.10	43.24	92.34	87.4	10.0	10.26.14	0.00
	18-Sep-99	swim	0.9	83028.20	52.94	98.02	87.4	10.0	10.48.31	0.00
	18-Sep-99	swim	1.1	82852.90	52.83	96.85	87.4	10.0	11.09.27	0.00
	18-Sep-99	swim	1.3	98316.10	62.69	101.80	87.4	10.0	11.29.46	0.00
	18-Sep-99	swim	0.0	52452.20	33.44	85.68	87.4	10.0	11.48.58	0.00
	18-Sep-99	swim	1.1	107547.80	68.57	111.80	87.4	10.0	12.14.38	0.00
	18-Sep-99	swim	0.0	59997.60	38.25	82.49	87.4	10.0	12.35.54	0.00
	18-Sep-99	swim	0.9	80506.80	51.33	92.75	87.4	10.0	12.54.25	0.00
	18-Sep-99	swim	1.1	91613.70	58.41	96.53	87.4	10.0	13.15.38	0.00
	18-Sep-99	swim	1.3	72975.70	46.53	99.84	87.4	10.0	13.35.09	0.00

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	fH ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Female 1	18-Sep-99	swim	0.00	46394.30	29.58	77.15	87.4	10.0	14.02.27	0.00
	18-Sep-99	swim	0.92	61457.40	39.18	89.71	87.4	10.0	14.23.35	0.00
	18-Sep-99	swim	1.11	85880.10	54.76	98.49	87.4	10.0	14.44.13	0.00
	20-Sep-99	swim	0.00	91077.10	58.07	114.42	87.4	10.0	09.05.21	0.00
	20-Sep-99	swim	0.92	107416.80	68.49	121.52	87.4	10.0	09.24.24	0.00
	20-Sep-99	swim	0.92	119289.80	76.06	114.45	87.4	10.0	09.42.02	0.00
	20-Sep-99	swim	0.00	53793.90	34.30	93.92	87.4	10.0	10.09.50	0.00
	21-Sep-99	swim	0.00	104861.70	66.86	118.42	87.4	10.0	09.26.36	0.20
	21-Sep-99	swim	0.00	82998.90	52.92	97.66	87.4	10.0	09.43.46	0.20
	21-Sep-99	swim	0.92	91398.10	58.28	105.06	87.4	10.0	10.02.54	0.20
	21-Sep-99	swim	0.92	77514.40	49.42	107.61	87.4	10.0	10.20.46	0.20
	21-Sep-99	swim	0.92	94728.90	60.40	108.39	87.4	10.0	10.38.26	0.20
	21-Sep-99	swim	0.00	63474.10	40.47	99.45	87.4	10.0	10.58.07	0.20
	21-Sep-99	swim	0.00	77651.10	49.51	90.69	87.4	10.0	11.16.48	0.20
	21-Sep-99	swim	0.92	72372.90	46.14	100.31	87.4	10.0	11.35.53	0.20
	21-Sep-99	swim	0.92	72101.70	45.97	91.41	87.4	10.0	11.53.33	0.20
	21-Sep-99	swim	0.00	56794.30	36.21	76.96	87.4	10.0	12.12.24	0.20
	21-Sep-99	swim	0.92	76254.60	48.62	101.00	87.4	10.0	12.33.06	0.20
	21-Sep-99	swim	0.92	107966.70	68.84	108.57	87.4	10.0	12.51.05	0.20
	25-Sep-99	swim	0.00	100158.40	63.86	110.86	87.4	10.0	08.58.40	0.10
	25-Sep-99	swim	0.00	95556.10	60.93	114.47	87.4	10.0	09.15.57	0.10
	25-Sep-99	swim	0.92	108364.50	69.09	107.11	87.4	10.0	09.35.32	0.10
	25-Sep-99	swim	0.92	107521.00	68.55	97.42	87.4	10.0	09.52.43	0.10
	25-Sep-99	swim	0.92	95969.10	61.19	92.53	87.4	10.0	10.09.59	0.10
	25-Sep-99	swim	0.00	60161.80	38.36	84.21	87.4	10.0	10.34.30	0.10
	25-Sep-99	swim	0.92	98924.50	63.07	102.38	87.4	10.0	10.52.19	0.10
	25-Sep-99	swim	0.00	67790.40	43.22	78.06	87.4	10.0	11.10.01	0.10
	25-Sep-99	swim	0.92	83546.10	53.27	94.16	87.4	10.0	11.28.03	0.10
	25-Sep-99	swim	0.00	62733.80	40.00	76.86	87.4	10.0	11.48.42	0.10

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Female 1	25-Sep-99	swim	0.92	76866.30	49.01	91.74	87.4	10.0	12.10.50	0.10
	25-Sep-99	swim	1.11	107176.80	68.34	97.75	87.4	10.0	12.29.30	0.10
	25-Sep-99	swim	1.11	94611.20	60.32	87.43	87.4	10.0	12.47.18	0.10
	25-Sep-99	swim	1.11	84309.40	53.76	93.56	87.4	10.0	13.04.39	0.10
	25-Sep-99	swim	1.30	92500.70	58.98	86.36	87.4	10.0	13.22.42	0.10
	25-Sep-99	swim	0.00	54044.80	34.46	73.78	87.4	10.0	13.43.59	0.10
	25-Sep-99	swim	1.11	94889.00	60.50	95.10	87.4	10.0	14.03.11	0.10
	25-Sep-99	swim	1.30	93208.90	59.43	90.28	87.4	10.0	14.22.02	0.10
	25-Sep-99	swim	1.30	86146.20	54.93	85.44	87.4	10.0	14.39.49	0.10
	25-Sep-99	hold	N/A	62289.90	39.72	82.66	87.4	10.0	14.51.46	0.10
	27-Sep-99	dry	N/A	95768.90	61.06	95.37	87.4	20.4	11.58.48	0.10
	28-Sep-99	dry	N/A	105814.80	67.47	96.03	87.4	21.8	10.45.05	0.00
Female 2	8-Jun-99	swim	0.00	96349.50	51.53	87.99	111.2	10.5	12.13.54	0.00
	8-Jun-99	swim	1.11	97758.98	52.28	91.78	111.2	10.5	12.35.18	0.00
	6-Nov-99	swim	0.00	52418.80	27.60	73.53	113.6	10.0	09.16.14	0.20
	6-Nov-99	swim	0.00	60671.70	31.95	76.29	113.6	10.0	09.35.25	0.20
	6-Nov-99	swim	0.92	79786.50	42.01	84.11	113.6	10.0	09.55.30	0.20
	6-Nov-99	swim	0.92	83324.20	43.87	83.10	113.6	10.5	10.13.12	0.20
	6-Nov-99	swim	0.00	67326.50	35.45	74.38	113.6	10.5	10.31.23	0.20
	6-Nov-99	swim	0.00	62446.20	32.88	68.47	113.6	10.0	10.49.35	0.20
	6-Nov-99	swim	0.92	80687.90	42.48	84.18	113.6	10.5	11.10.55	0.20
	6-Nov-99	swim	0.92	72963.90	38.42	78.55	113.6	10.5	11.28.37	0.20
	6-Nov-99	swim	0.00	56493.50	29.75	70.16	113.6	10.5	11.46.50	0.20
	6-Nov-99	swim	0.92	73972.80	38.95	75.17	113.6	10.5	12.05.19	0.20
	6-Nov-99	swim	1.11	86586.70	45.59	80.10	113.6	10.5	12.26.46	0.20
	6-Nov-99	swim	0.00	54226.60	28.55	65.42	113.6	10.5	12.45.03	0.20
	6-Nov-99	hold	0.00	78214.50	41.18	87.17	113.6	10.5	12.53.43	0.20
	16-Nov-99	swim	0.00	86429.60	44.99	88.45	115.4	10.0	09.12.28	0.20
	16-Nov-99	swim	0.00	74908.60	38.99	84.60	115.4	10.0	09.30.10	0.20

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Female 2	16-Nov-99	swim	0.0	95269.20	49.59	88.71	115.4	10.0	09.48.00	0.20
	16-Dec-99	swim	0.0	79415.88	41.65	77.31	114.2	9.0	10.17.52	0.15
	16-Dec-99	swim	0.0	63115.13	33.10	71.58	114.2	9.0	10.35.03	0.15
	16-Dec-99	swim	0.0	26956.97	14.14	58.34	114.2	9.0	10.54.14	0.15
	16-Dec-99	swim	0.9	77632.63	40.72	76.76	114.2	9.5	11.15.23	0.15
	16-Dec-99	swim	0.0	69699.02	36.56	71.89	114.2	9.5	11.35.29	0.15
	16-Dec-99	swim	0.9	79952.30	41.94	73.89	114.2	9.5	11.55.30	0.15
	16-Dec-99	swim	0.9	79830.13	41.87	72.55	114.2	9.5	12.14.07	0.15
	16-Dec-99	swim	0.9	75873.26	39.80	73.99	114.2	9.5	12.32.45	0.15
	16-Dec-99	swim	0.9	80150.88	42.04	71.42	114.2	9.5	12.51.45	0.15
	16-Dec-99	swim	0.0	47705.91	25.02	60.86	114.2	9.5	13.11.30	0.15
	16-Dec-99	swim	0.9	68253.55	35.80	68.08	114.2	9.5	13.31.35	0.15
	16-Dec-99	swim	0.9	66456.44	34.86	61.86	114.2	9.5	13.49.53	0.15
	20-Dec-99	swim	0.0	86956.41	45.49	84.98	114.6	9.5	10.46.38	0.10
	20-Dec-99	swim	0.0	73321.22	38.36	73.62	114.6	9.5	11.05.15	0.10
	20-Dec-99	swim	0.9	101229.90	52.96	76.21	114.6	9.5	11.27.11	0.10
	20-Dec-99	swim	0.9	95245.43	49.83	72.48	114.6	9.5	11.45.38	0.10
	20-Dec-99	swim	0.9	83198.00	43.53	69.06	114.6	9.5	12.07.28	0.10
	20-Dec-99	swim	0.0	81955.20	42.88	69.15	114.6	9.5	12.28.05	0.10
	20-Dec-99	swim	0.0	84064.02	43.98	73.59	114.6	10.0	12.47.15	0.10
	20-Dec-99	swim	0.9	86805.56	45.41	67.55	114.6	10.0	13.07.41	0.10
	20-Dec-99	swim	0.9	82937.52	43.39	65.60	114.6	10.0	13.26.01	0.10
	20-Dec-99	swim	0.9	84551.48	44.23	62.42	114.6	10.0	13.46.02	0.10
	20-Dec-99	swim	0.9	80800.16	42.27	64.62	114.6	10.0	14.07.08	0.10
	20-Dec-99	swim	0.0	80260.23	41.99	66.43	114.6	10.0	14.27.05	0.10
	20-Dec-99	hold	0.0	63746.22	33.35	65.82	114.6	10.0	14.35.54	0.10
	22-Dec-99	swim	0.0	112441.80	58.98	86.90	114.2	9.0	11.13.43	0.20
	22-Dec-99	swim	0.0	62318.66	32.69	67.58	114.2	9.0	11.32.06	0.20
	22-Dec-99	swim	1.1	96163.34	50.44	70.71	114.2	9.0	11.51.14	0.20

Table A1.1 cont.

Animal	Date	Trial Type	Water Current Speed ($m \cdot s^{-1}$)	Total VO_2 ($L \cdot hr^{-1}$)	VO_2 ($ml \cdot min^{-1} \cdot kg^{-0.75}$)	f_H ($beats \cdot min^{-1}$)	Animal Mass (kg)	Temperature (°C in water or chamber)	Time of Day (hh.mm.ss)	Food Ingested (kg) Within Last 12 Hours
Female 2	22-Dec-99	swim	1.1	95822.98	50.26	67.31	114.2	9.0	12.09.23	0.20
	22-Dec-99	swim	1.1	82219.11	43.12	64.92	114.2	9.5	12.27.54	0.20
	22-Dec-99	swim	0.0	90702.04	47.57	74.84	114.2	9.5	12.48.40	0.20
	22-Dec-99	swim	0.0	87280.30	45.78	64.42	114.2	9.5	13.07.08	0.20
	22-Dec-99	swim	1.1	83821.45	43.97	61.73	114.2	9.5	13.27.11	0.20
	22-Dec-99	swim	1.1	84105.84	44.11	64.41	114.2	9.5	13.46.36	0.20
	22-Dec-99	hold	0.0	62350.67	32.70	75.31	114.2	9.5	14.03.56	0.20

Appendix 2: Electrode Design and Summary of Performance

Over the course of the project, several electrode designs were used to collect ECG data with varying results. The following documents and evaluates the four different types used: a surface disk electrode, and three styles of subcutaneous electrodes.

Surface disk electrodes

In the first phase of the experiment, October 1998 to January 1999, rigid surface disk electrodes were constructed and glued to the fur of Male 1 and later to Male 2.

Construction: Each electrode consisted of a base made from Sealtronic epoxy encasing resin (Industrial Formulators of Canada, Ltd., British Columbia) and a central modified silver coin for ECG detection.

To construct the base, a female square mini-conn underwater connector (Underwater Systems, California) was hot-glued to the centre of a 10-centimetre watchglass-shaped mold previously brushed with a releasing compound. All entrances into the connector were blocked with hot-glue. The wire tail of the connector was bent to rise up out of the mold. The epoxy was then poured into the mold, completely encasing the connector and part of the wire, and filling the mold to a height of 1 centimetre. Once the epoxy base was hardened, it was removed from the mold.

The upturned wire was cut to within 3 centimetres of the epoxy and the centimetre from its tip was stripped of neoprene. The entire section of free wire was wrapped around a centimetre-high silver stake that was previously welded perpendicularly to a filed-smooth 80% fine silver quarter. The stripped tip of the wire was soldered to the coin.

After soldering, the stake was inserted within a hole drilled through the centre of the hardened epoxy base, leaving a few millimetres of space between the base and the coin. A small amount of CIBA Fastweld 10 Epoxy (Ciba-Geigy Corp., Michigan) was inserted into this space to secure the coin to the centre of the base.

To complete the electrode (Fig. A2.1), sharp edges of epoxy were ground smooth and the entrance to the embedded female connector was drilled open.

Application: In preparation for attaching the disk electrodes to the animal, the fur was dried and rubbed free of its oils with acetone on one shoulder and on the opposing side just above the pelvis (Fig. 1.1). A circular patch (about 2.5 centimetres in diameter) was shaved free of fur in the cleaned area with motorized clippers and disposable razors cut to size.

Tenset epoxy (Fibreglass-Evercoat Co., Inc., Ohio) was applied to each electrode disk in a ring around the central coin. Epoxy was also applied to the fur in a ring around each shaved patch. A dab of electrode gel was placed on each coin's surface. The disk was pressed firmly against the fur of the animal, taking care to match the coin's surface to the shaved patch and the ring of epoxy on the fur to that on the disk. The female connectors in the disk were then hooked to matching male connectors from the datalogger.

Summary of Performance: The length of time that the disk electrodes remained on the animal varied from 12 to 28 days, which, in the early phase of the project, was too little time to collect the required data.

When the animal flexed his body, the rigid disks did not bend with the animal's skin. Strain from such flexing eventually led to fur being torn from the skin and the

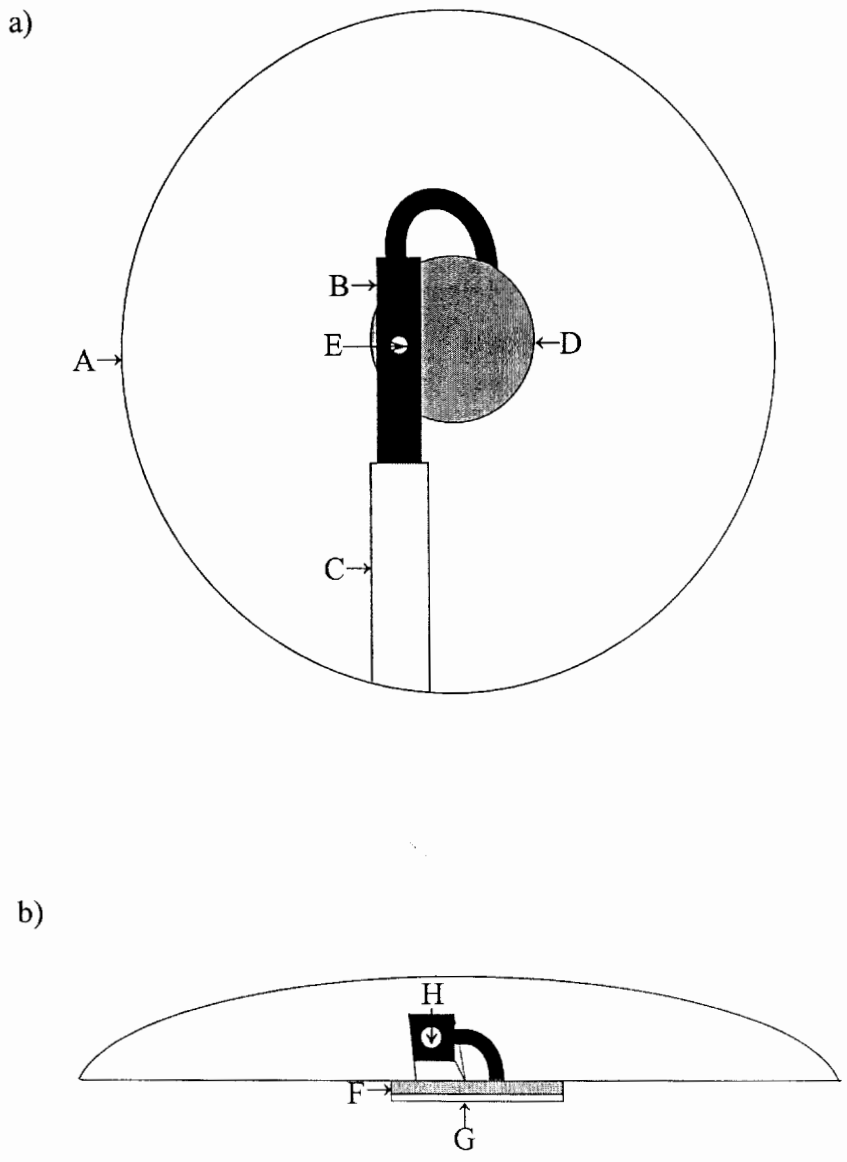

Fig. A2.1. Surface disk electrode. a) Top view looking down through transparent epoxy and b) side view. A, epoxy disk; B, female connector; C, groove through epoxy to connector entrance; D, silver coin; E, water-expulsion hole; F, CIBA epoxy; G, silver coin; H, entrance into female connector.

edges of the disks becoming free. Once this happened, the edges were easily snagged on enclosure fences or an animal could grab hold of the disks with its teeth. These, coupled with the lack of epoxy in the centre of the disk (which would anchor the disk to the animal, but block ECG pickup), reduced the length of time the disks remained attached to the body. On male 1, the shoulder electrode was replaced twice while the pelvic electrode was replaced once. Male 2 had his pelvic electrode replaced once. Removal of electrodes also meant removal of fur, thus making it more difficult to glue another electrode.

A clean ECG was never obtained from either male when swimming in the mill with surface disks. It is suspected that the electrodes were not completely sealed off from the seawater. This caused a short circuit between the two disks, reducing their difference in electric potential. Thus, flatlines (no clear ECG) were usually obtained during swim trials.

Subcutaneous electrodes- Style 1

With the inherent problems with the disks, subcutaneous electrodes were designed and tested.

Construction: The copper wire tail of a female underwater connector was soldered to one end of a twelve-centimetre section of 28-gauge, bioelectric cable (Cooner Wire, California). This cable consisted of several layers: an inner stranded stainless steel conductor coated with Teflon and an outer stainless steel braided shield coated with PVC. Only the innermost steel conductor was soldered to the connector's wire.

Before insertion, a centimetre of the free end of the cable was stripped of all but its innermost conductor, and the entire cable was sterilized.

Application: The fur was rubbed dry and free of oils with acetone. A small patch of fur (about 5 mm in diameter) was shaved with either an electric razor or small scissors. This allowed a clear marker of where the needle was to be inserted. To assist in inserting the needle through the tough skin of the animal, a #11 scalpel blade was used to make a preliminary cut about 3-millimetres in length through the skin. Any resulting blood was absorbed with dry sterile gauze.

All sub-cutaneous electrode insertions and scalpel cuts were performed by Dr. David Huff, the veterinarian at the Vancouver Aquarium Marine Science Centre.

The stripped section along with a few millimetres of the following unstripped bioelectric cable was inserted into the shaft of a 13-gauge reusable hypodermic needle (Popper and Sons, New York). Inserting some of the unstripped section prevented the bevel from cutting the fragile inner wires during folding. The rest of the insulated cable was folded over the end of the needle and held parallel against it. While the cable and the epoxy base were held close to the needle, the needle (with the cable inserted into the bevel) was inserted firmly into the skin through the cut made with the scalpel blade. It was guided subcutaneously as parallel to the skin as possible to avoid underlying blubber, and was inserted until its hub hit the outer surface of the animal. The needle was then gently removed from the animal while the cable was held in place, preventing its removal from under the skin. Again, any blood released during the procedure was absorbed immediately with dry, sterile gauze to ensure a clean, dry gluing surface for the next step.

All that remained of the electrode above the skin after insertion was the female connector. Aside from the main entrance for the male connector, each female connector had been manufactured with a small secondary hole on one side to allow water trapped inside it to exit freely as the male connector was inserted. With an easily removable wooden plug inserted inside the hole, the connector was glued (with the expulsion hole facing up) with Tenset epoxy to the nearby fur of the animal, taking care not to fill in the connector's entrance. The final result was a connector almost completely buried in epoxy, held solidly to the fur (Fig. A2.2).

Summary of Performance: Although they had the drawback of being invasive, subcutaneous electrodes occupied a significantly smaller area on the fur of the animal, thus reducing the loss of fur when the electrodes were finally removed. The edges of the epoxy continued to catch on objects in the animals' environment. However, once the edges lifted up from the fur, the connectors remained solidly in position because they were anchored by a continuous epoxy base. There was no central contact point free of glue, as was the case with the disk electrodes. The connectors remained attached to the animal well into a second month. However, the entire gluing procedure was messy and inefficient.

With the invasiveness of the procedure came irritation and signs of infection, which became a substantial health concern. Pus, heat, and redness were commonly observed in the area surrounding the wire's insertion. Possibly due to irritation, the animal often chewed at the base of the connector where the wire was joined, and pulled the wire from its skin. In other instances, the wire gradually worked its way out of the

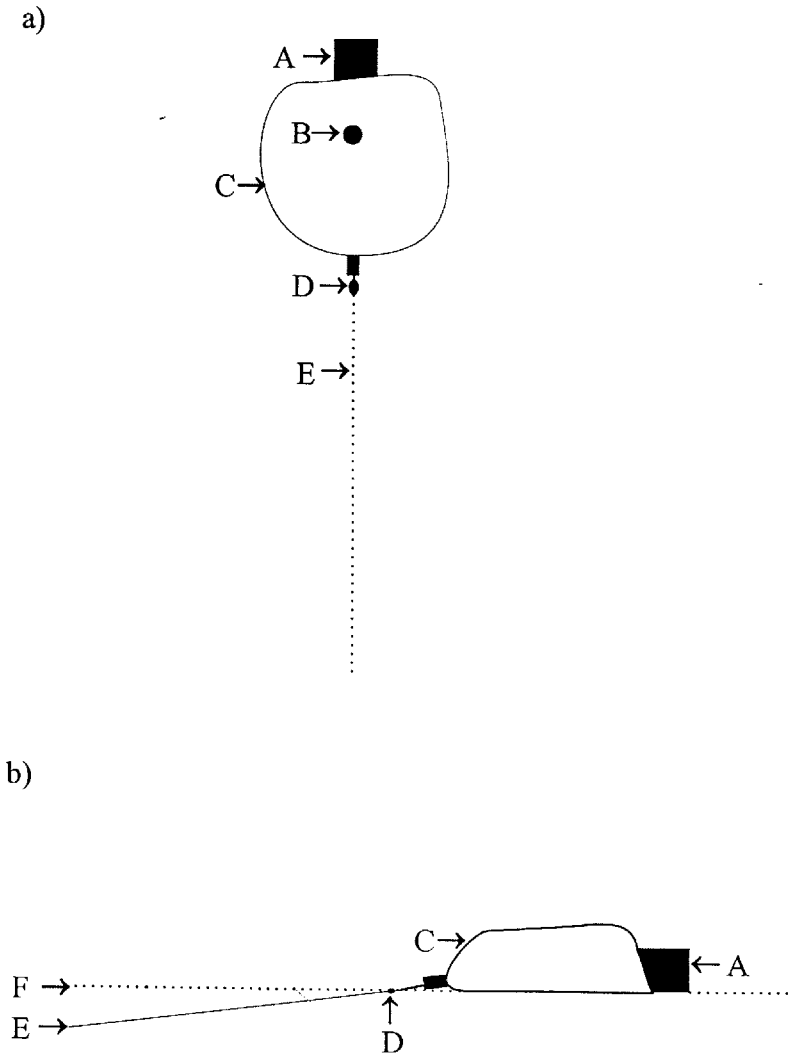

Fig. A2.2. Subcutaneous electrode style 1. a) Top view and b) side view. A, buried female connector; B, water expulsion hole; C, Ten Set epoxy; D, insertion point through skin; E, subcutaneous bioelectric wire; F, skin surface.

skin naturally as the animal flexed, forming a loop for the animal to easily grab at. Wires remained in the skin for only 2-15 days; again, not long enough to obtain the necessary data.

Beginning two days prior to subsequent electrode insertion and continuing for the following one or two weeks, each glued animal was administered 4-5 68 mg tablets (or 2.5-3 150 mg tablets) twice daily of Baytril, an antibiotic to decrease the risk of infection. They also received 2-3 100 mg tablets twice daily of Rimadyl, an anti-inflammatory to decrease the irritation. Once this procedure was adopted, the animals appeared less interested in biting at the electrodes and pus was rarely reported.

Subcutaneous electrodes- Style 2

The second style of subcutaneous electrode was developed in hopes of reducing application time and difficulty of gluing the base to the fur.

Construction: A female underwater connector was soldered to the innermost steel conductor of the bioelectric cable as described above. A section of neoprene with one rubber surface and one nylon-coated surface was then cut into a 3.5-centimetre square. The connector was hot-glued into the middle of the neoprene patch on the nylon surface with its water-expulsion hole facing up (Fig. A2.3). Both the water-expulsion hole and the main entrance to the connector were temporarily covered with hot glue to prevent epoxy from filling the connector, rendering it useless.

A wall of Fun Tak was molded around the edge of the neoprene surrounding the connector and the solder joint so that the epoxy would completely cover both, and roughly take the form desired for the rigid base of the electrode. To reduce strain in the

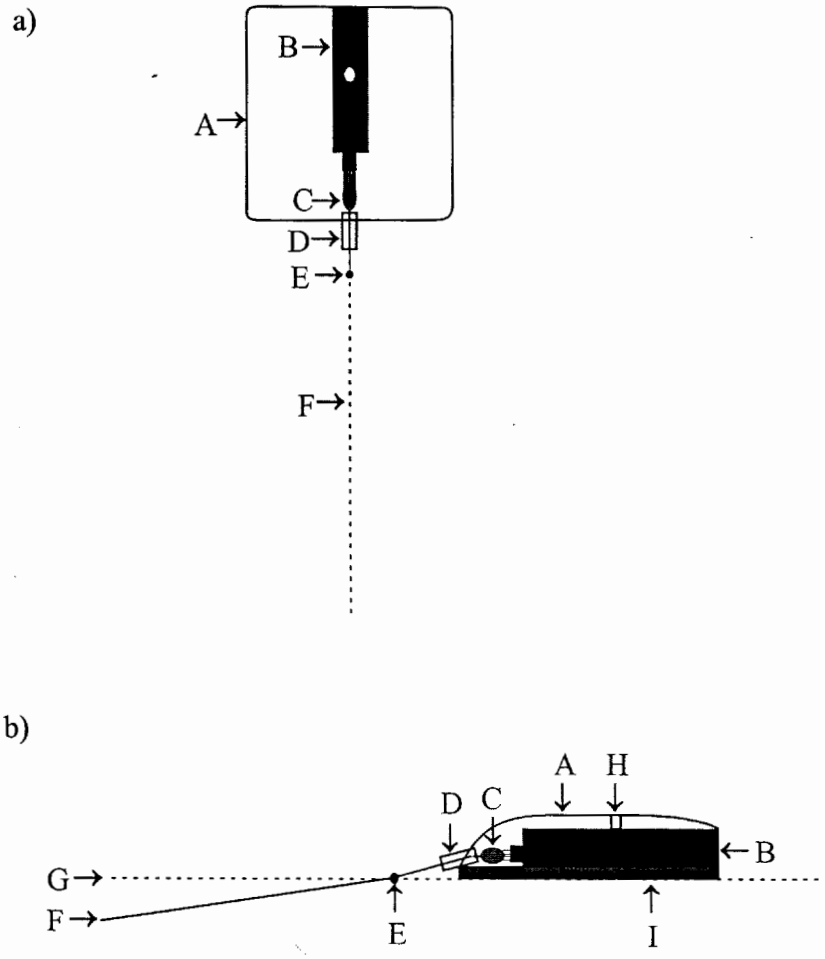

Fig. A2.3. Subcutaneous electrode style 2. a) Top view looking through transparent epoxy and b) side view through epoxy. A, epoxy block; B, female connector; C, solder joint; D, Tygon tubing; E, insertion point through skin; F, subcutaneous wire; G, skin surface; H, water expulsion hole drilled through epoxy; I, neoprene patch.

area where the wire was to come out of the epoxy, a small section of Tygon tubing was positioned over the wire. This resulted in half the tubing being positioned inside the wall and half remaining outside. The tubing was secured within the epoxy at one end, but gave some protection and flexibility to the wire where it was came out of the epoxy. The Fun Tak was also used to completely block the entrance of the connector to prevent any influx of epoxy.

The Fun Tak mold was slowly filled with epoxy until the connector was covered with a few millimetres in depth. When the epoxy appeared solidified after about 10 minutes, the Fun Tak was easily peeled away and the hot-glue from the entrance to the connector was also removed.

After 24 hours, the epoxy was rigid enough for grinding to the shape desired using a high-speed rotary moto-tool (Dremel, Wisconsin). Sharp edges were removed and the entire epoxy base was ground as flat as possible to prevent the electrode from snagging on surrounding objects once glued onto the animal. The water-expulsion hole was also drilled clear of any overlying epoxy to allow water to flow freely through the connector. The inside of the connector was cleaned with a pipe cleaner sprayed with WD-40. Each electrode was tested for zero resistance with an Ohmmeter.

Application: Insertion of the wire is described above in Subcutaneous Style 1. Just before insertion, the rubber neoprene underside of the electrode was cleaned with a Cyano-Prep solution.

When the fur was dry and free of blood, drops of Loctite were applied and spread evenly over the rubber underside of the neoprene and over the fur in the area where the neoprene was to be attached. The neoprene was pressed to the fur with the glued surfaces

matching as closely as possible, ensuring that the insertion point remained uncovered to allowing constant flushing of the wound with seawater (Fig. A2.3). Once the Loctite dried, a clean ECG pickup was tested with a 50 MHz Fluke 97 Scopemeter (John Fluke Mfg. Co., Washington).

After completion of trials, the neoprene was cut with a scalpel blade to remove most of the electrode base and the entire cable from the animal.

Summary of Performance: With the previously-hardened epoxy base, gluing the connectors to the animal with fast-setting Loctite was completed with less mess and in far less time then the initial subcutaneous style. Again, the bases of the electrodes remained solidly glued to the fur for one to two months. The neoprene layer between the epoxy and the fur could easily be cut through to remove the electrode if problems arose (ie: if the wire lifted prematurely) Infection and irritation remained under control with the administered antibiotic and anti-inflammatory.

However, the problem with the wire eventually rising out of the skin under pressure of the animal's flexing remained unsolved, hence, the development of the mesh overlay and surrounding neoprene design of the final subcutaneous electrode.

Subcutaneous electrodes- Final Style

Construction: A female underwater connector was soldered to one end of a twelve-centimetre section of 28-gauge, bioelectric cable as described in Subcutaneous Style 1. A small piece of the rubber- and nylon-coated neoprene was then cut to the desired shape and size (Fig. A2.4). The connector was hot-glued into the proper position on the nylon surface of the neoprene with the water-expulsion hole facing up. Both the water-

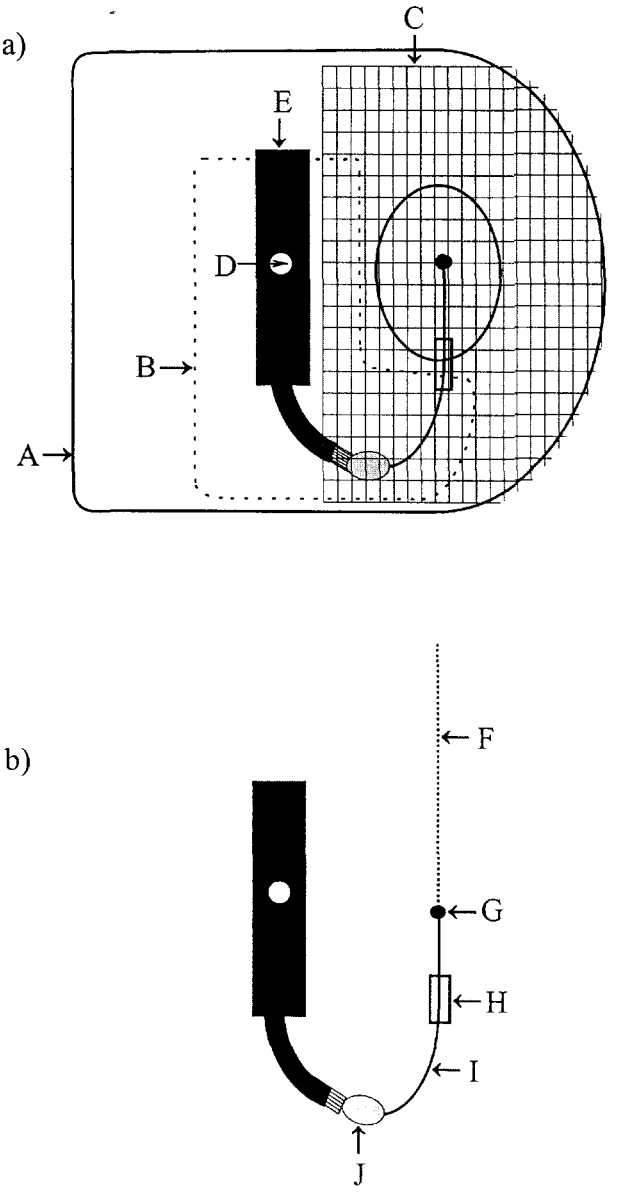

Fig. A2.4. Subcutaneous electrode, final style. a) Top view, b) top view with mesh and neoprene base removed. A, neoprene base; B, edge of epoxy; C, mesh overlay; D, water-expulsion hole; E, entrance into female connector; F, subcutaneous bioelectric wire; G, insertion hole in skin; H, Tygon tubing; I, bioelectric wire inside epoxy base; J, solder joint inside epoxy base.

expulsion hole and the entrance to the connector were temporarily covered with hot glue to prevent epoxy from filling the connector. Tygon tubing was positioned over the wire as described in Subcutaneous Style 2. A cut-to-fit piece of plastic mesh was hot-glued to the section of neoprene between the connector and the hole through which the wire would run. The mesh would later assist in preventing the wire from lifting out once it was inserted under the skin.

A wall of Fun Tak was molded on the nylon surface of the neoprene around the connector and the solder joint so that the epoxy would completely cover both and roughly take the form desired for the rigid base of the electrode. The area to be immersed in epoxy was kept as small as possible (the epoxy did not cover the entire section of neoprene) to maintain some flexibility of the neoprene to ease wire insertion, but was large enough to ensure a watertight seal around the solder joint and to allow a solid base for gluing to the animal. As before, the Fun Tak was molded to completely cover the front entrance of the connector to prevent any influx of epoxy. It was also positioned in such a way as to allow only the side of the mesh between the connector and the hole in the neoprene to be immersed in epoxy. The main body of the mesh was left free.

Pouring and preparing the epoxy was the same as for Subcutaneous Style 2.

Application of electrodes: The cable was inserted as described for Subcutaneous Style 1. While the cable was being inserted with the needle, the flap of neoprene under the free mesh could be folded up to ease the process. The neoprene was cleaned and then glued to the fur as described for Subcutaneous Style 2. When the neoprene was pressed to the fur, the cable insertion point lay directly under the hole in the neoprene, free from glue. This allowed flushing of the wound with seawater, reducing the chance of infection and

allowing early visual detection should an infection occur. A piece of cloth cut to the proper shape and size of the neoprene surrounding the insertion hole was laid over and glued with Loctite over the free edge of the mesh, securing the mesh to the neoprene. Both electrodes were tested with the Scopemeter.

Electrodes were removed as described for Style 2.

Summary of Performance: These electrodes were the most successful of the styles tried. Both the base of the electrode and the subcutaneous wire remained properly in place for as long as was necessary (more than a month). With practise, the entire insertion and gluing process was completed within 20 minutes. Infection and irritation remained under control with the administered antibiotic and anti-inflammatory. The animals did not bite at the electrodes.

Appendix 3: Spring-Clip and Eye Attachment System

A spring-clip and eye system for the attachment of the datalogger to the animal was designed as an alternative to wearing the datalogger in a harness (Fig. A3.1). Two spring-clips glued to the logger could be quickly snapped into two loops ("eyes") glued to the animal's fur.

To construct the eye of the system, five holes were cut through a quarter. The legs of two stainless steel cotter pins were cut down so that the heights of the pins were no longer than a centimetre. The legs of the resulting loops were soldered to the quarter so that the tops of the loops were leaning against each other above the large hole in the centre of the quarter.

Before attaching the eyes, two strips of Velcro were glued with Loctite Quickset Rubber Bonder (Loctite Corporation, Ontario) to the fur of the animal. Two opposing strips were glued to the underside of the logger. The logger could then be held in place along the midline of the dorsal surface using the Velcro attachments.

Two quarters modified in the above manner were attached to the fur of the animal with Five-minute epoxy (ITW Devcon, Massachusetts). One quarter was placed onto the fur at a distance of 6cm from the head of the logger and the other 6 centimetres away from its foot. A 0.5cm-high plastic ring with a diameter of about 3.5cm was placed over each quarter and was used to dam the flow of the epoxy when poured. The holes in the quarters allowed the epoxy to flow up through and onto the quarters' surface, firmly holding them in place once it hardened. To avoid filling the passageway beneath the cotter pin loops with epoxy, the quarter was held at the surface of the epoxy while it was poured and a small pin was passed repeatedly through the passage during hardening.

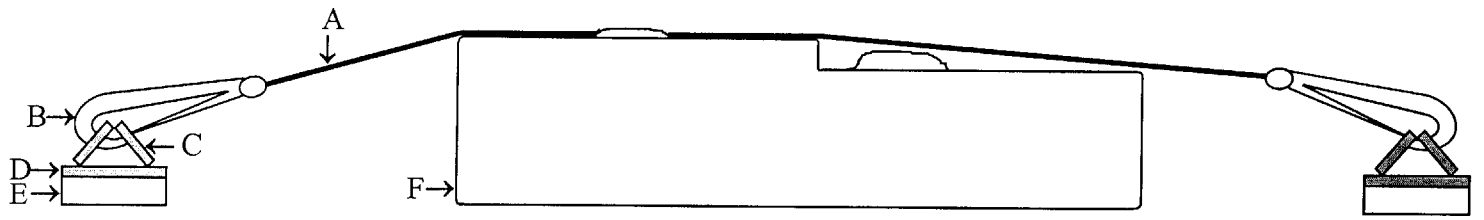

Fig. A3.1 Spring-clip and eye datalogger attachment system. A, elastic band; B; spring-clip; C, modified cotter pin; D, quarter; E, epoxy base; F, datalogger.

When possible, the plastic ring was removed so that only a small disk of epoxy with a quarter sitting on its top surface was left on the fur.

A fabric-coated elastic band 2.5cm in width was glued in one spot onto the top surface of the logger so that it extended along the logger's length. One 3.5cm-long brass spring clip was attached to each end of the elastic. When the logger was placed into position with the Velcro, each clip was then clipped through the cotter pin loops on the corresponding quarter. The logger could easily be attached and removed from the animal with this system.

Appendix 4: The Effects of Feeding on the Relationship between Heart Rate and Oxygen Consumption in a Captive Juvenile Steller Sea Lion

Introduction

Relationships between heart rate (f_H) and oxygen consumption ($\dot{V}O_2$) have been reported for a number of species, suggesting that energy expenditure of free-ranging animals may be predicted from heart rate recordings (Fedak, 1986; Williams et al., 1991; Bevan et al., 1992; Nolet et al., 1992; Butler, 1993; Williams et al., 1993; Bevan et al., 1994; Bevan et al., 1995a; Bevan et al., 1995b; Boyd et al., 1995; Hurley, 1996). However, studies conducted to date have not investigated whether the reliability of this technique is affected by the ingestion of food.

As observed by Lavoisier in the 18th century and described later in 1877 as the "work of digestion" by Bidder and Schimdt, oxygen consumption generally increases after a quantity of food is ingested (Kleiber, 1961). This phenomenon is often referred to as the heat increment of feeding or specific dynamic action (Blaxter, 1989; Rosen and Trites, 1997). Feeding can also invoke changes in haemodynamic variables such as heart rate, stroke volume and cardiac output to meet increased blood flow requirements of the gut for digestion. The nature and duration of these postprandial changes varies greatly among studies. Heart rate does not consistently increase, and even if it does increase, it may not be a major contributor to the enhanced blood flow (Grollman, 1929; Gladstone, 1935; Kelbaek et al., 1989; Waaler et al., 1991; Muller et al., 1992; Sidery and MacDonald, 1994; Kearney et al., 1995). Thus, heart rate may not always be a good estimator of oxygen consumption (or energy expenditure) following feeding in some species.

To my knowledge, no studies have examined how feeding may change the $f_H/\dot{V}O_2$ regression. Instead, the $f_H/\dot{V}O_2$ relationship has been determined for subjects fasted prior to any experiments (thus, excluding food effects completely) or determined after the subjects had the opportunity to ingest a meal (excluding comparison between data with and without food) (Williams et al., 1991; Butler, 1993; Li and Hautvast, 1993; McCrory et al., 1997). Only one study on bottlenose dolphins (Williams et al., 1993) and one on Antarctic fur seals (Boyd et al., 1999) have determined the $f_H/\dot{V}O_2$ relationship over a period that most likely involved some feeding. Close relationships between f_H and $\dot{V}O_2$ were reported in these studies, but whether or not feeding changed the relationship was not tested. In other marine mammal studies (all in captivity), animals were fasted prior to trials.

As seen in Chapter 1, experiments conducted with fasted, captive Steller sea lions have found significant relationships between f_H and $\dot{V}O_2$. Apart from the breeding season, during which many male Steller sea lions fast for 2-3 months (Riedman, 1990), free-ranging sea lions would likely be feeding at times throughout any datalogger deployment period. Therefore, the next logical step in investigating the application of the heart rate method to free-ranging animals was to determine whether feeding affects these relationships. The following outlines a series of repeated trials conducted on a single Steller sea lion to further examine the accuracy of estimating energy expenditure from heart rate over periods of time that encompass feeding events.

Materials and Methods

Data were collected between May 4 and June 3, 2000 from one three-year-old male Steller sea lion (Male 2 from the previous experiment; mass 194.4 kg) housed at the Vancouver Aquarium Marine Science Centre with an environment and husbandry protocol as described in Chapter 1. Only one animal was studied due to time constraints and because electrodes remained attached to this animal well after the completion of the fasting study (Chapter 1).

Experimental Apparatus

Monitoring heart rate: The electrocardiogram (ECG) was sampled and recorded by a datalogger at 100Hz from two dorsal subcutaneous electrodes positioned above one shoulder blade and above the pelvis on opposing sides (Fig. 1.1). Electrode design was as described under "final style" in Appendix 2 (Fig. A2.4). The logger was temporarily attached to the animal during feeding sessions using a spring-clip and eye system (Appendix 3, Fig. A3.1).

Monitoring oxygen consumption: For each feeding session, open circuit respirometry measured oxygen consumption of the sea lion while he swam in the swim mill, as described in Chapter 1. Water temperature remained at 10°C.

Protocol for Feeding Sessions

Male 2 entered the swim mill 32 - 73 minutes (depending on behaviour) after having ingested a bulk amount of either 6 or 12 kg of herring. One feeding session consisted of 3 hours in total of monitoring the animal's heart rate and oxygen

consumption while swimming in the swim mill without a current. After an initial 10-minute air-equilibration phase, each session was split into successive trials lasting 5 minutes each. When one 5-minute trial ended, another began immediately following it until three hours had passed since the first trial. Three feeding sessions preceded by 6-kg feedings were performed on three separate days in close succession, followed by another three sessions after 12-kg feedings.

Analysis

Heart rate: The recorded ECG was downloaded from the datalogger after each feeding experiment using the computer communications software Crosscut Version 2.01 (Onset Computer Corp, 1994). The program Acknowledge 3.0 (Biopac Systems, Inc. and Microsoft Corp., 1992-1995) calculated interbeat intervals on the ECG, and from these, mean heart rate in beats·min^{-1} was calculated over each 5-minute feeding trial.

Oxygen consumption: Mean oxygen consumption was calculated from oxygen concentration and expressed in ml $O_2 \cdot min^{-1} \cdot kg^{-0.73}$ during the same 5-minute intervals as heart rate.

***f*H/ $\dot{V}O_2$ Relationships:** Using data from all 5-minute trials collected during the 6 feeding sessions, heart rate was regressed against oxygen consumption for Male 2. This regression was compared with the regression obtained when fasting previously reported in Chapter 1. For each of the 6 feeding sessions, a time series using mean heart rate from each 5-minute trial and a second using mean oxygen consumption were constructed to investigate behaviour of the variables as digestion progressed after feeding.

Results

Oxygen consumption significantly increased over time following consumption of all 6 and 12 kg meals (Fig. A4.1). However, heart rate was more variable and increased slightly in only one of the six trials (a 12 kg feeding). Heart rate either declined or showed no significant change as $\dot{V}O_2$ rose in all other 6 and 12 kg feeding trials (Fig. A4.1, Table A4.1). In general, however, higher mean heart rates and oxygen consumption values were associated with larger meals ($p<0.001$ with ANOVA and TUKEY tests comparing either heart rate or oxygen consumption after 0, 6 and 12 kg food; see Fig A4.2 for heart rates).

When the 12-kg feeding trial data were considered alone, $\dot{V}O_2$ changes were independent of f_H ($p=0.15$), while the 6-kg trials had only a weak linear $f_H/\dot{V}O_2$ relationship ($\dot{V}O_2 = (0.27 f_H \pm 0.04) + (15.98 \pm 3.29)$, $p<0.01$, $r^2=0.27$).

When all feeding data was considered, the relationship between f_H and $\dot{V}O_2$ was weak (Fig. A4.3, $p<0.01$, $r^2=0.19$) compared to when the animal was fasting (Fig. A4.4, $p<0.01$, $r^2 = 0.71$). These two regressions from Male 2 (i.e., 1. fasting trials only and 2. feeding trials only) were significantly different ($p<0.05$), indicating that digestion of food alters the $f_H/\dot{V}O_2$ relationship derived for fasted animals.

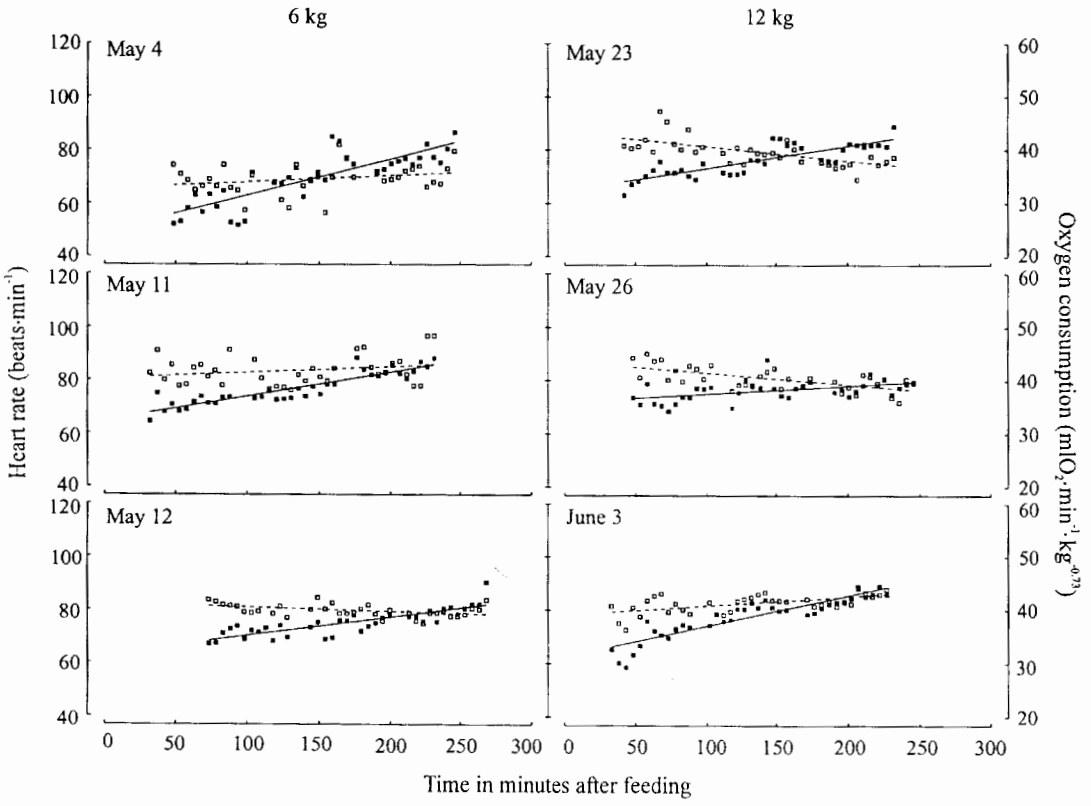

Fig. A4.1. Time series of heart rate (beats·min^{-1})(▫) and oxygen consumption (mlO_2·min^{-1}·kg$^{-0.73}$) (▪) in Male 2 after 6 and 12 kg feedings.

Table A4.1. *Regression parameters describing the linear change in* $\dot{V}O_2$ *over time following bulk feeding. Significant differences at the p<0.01 level are in bold.*

Date (2000)	Food Ingested (kg)	f_H			$\dot{V}O_2$		
		Regression	r^2	p	Regression	r^2	p
May 4	6	f_H= 0.02time + 65.30	0.07	0.11	$\dot{V}O_2$=0.07time + 24.61	0.71	**<0.001**
May 11	6	f_H= 0.02time + 80.49	0.06	0.15	$\dot{V}O_2$=0.04time + 32.33	0.82	**<0.001**
May 12	6	f_H=-0.02time + 82.33	0.16	**0.016**	$\dot{V}O_2$=0.03time + 31.53	0.70	**<0.001**
May 23	12	f_H=-0.05time + 86.55	0.40	**<0.001**	$\dot{V}O_2$=0.04time + 32.27	0.69	**<0.001**
May 26	12	f_H=-0.04time + 87.20	0.40	**<0.001**	$\dot{V}O_2$=0.02time + 35.87	0.25	**<0.001**
June 3	12	f_H= 0.04time + 78.09	0.35	**<0.001**	$\dot{V}O_2$=0.06time + 31.24	0.87	**<0.001**

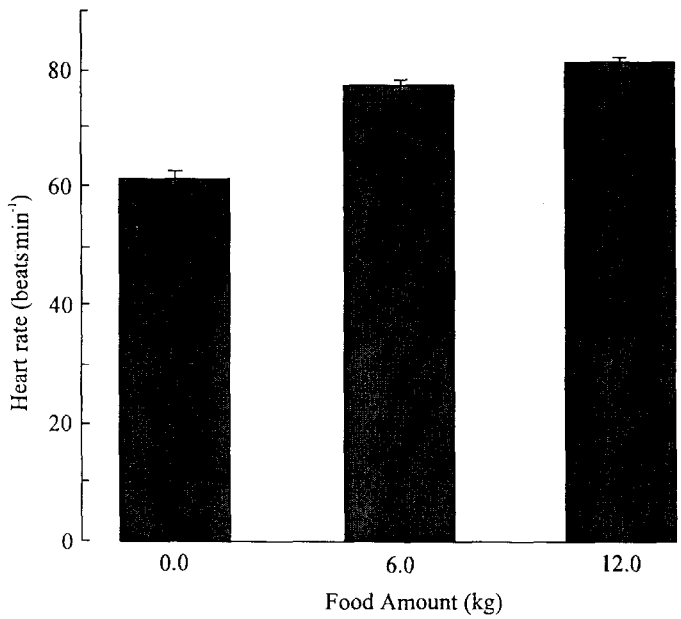

Fig. A4.2. Mean heart rate (beats·min^{-1}) recorded in the swimmill from Male 2 after fasting or ingesting 6.0 or 12.0 kg of herring. The heart rate plotted after the 6.0 or 12.0 kg meal is the postprandial mean heart rate of 18 feeding trials. The heart rate plotted without prior food (0.0 kg) is the mean of the fasting trials while the animal swam without a current (taken from Chapter 1). Bars indicate standard error of the means. Mean heart rate differed among all three feeding levels ($p<0.01$).

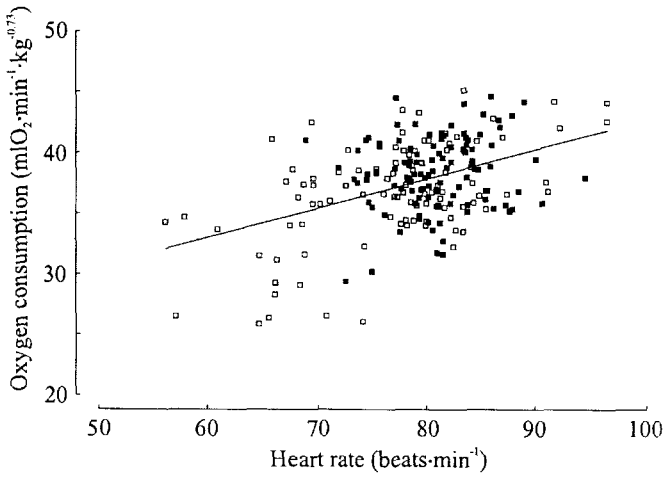

Fig. A4.3. Relationship between postprandial heart rate (beats·min^{-1}) and oxygen consumption (mlO_2·min^{-1}·kg$^{-0.73}$) as determined in male 2 after a bulk feed of 6 (▫) or 12 (▪) kg. Data points, consisting of 5-minute mean f_H and $\dot{V}O_2$ values, were collected between 30 minutes and 4 hours after feeding. The regression equation for the pooled data is $\dot{V}O_2 = (0.24 f_H \pm 0.03) + (18.49 \pm 2.68)$, $r^2 = 0.19$, $p<0.01$.

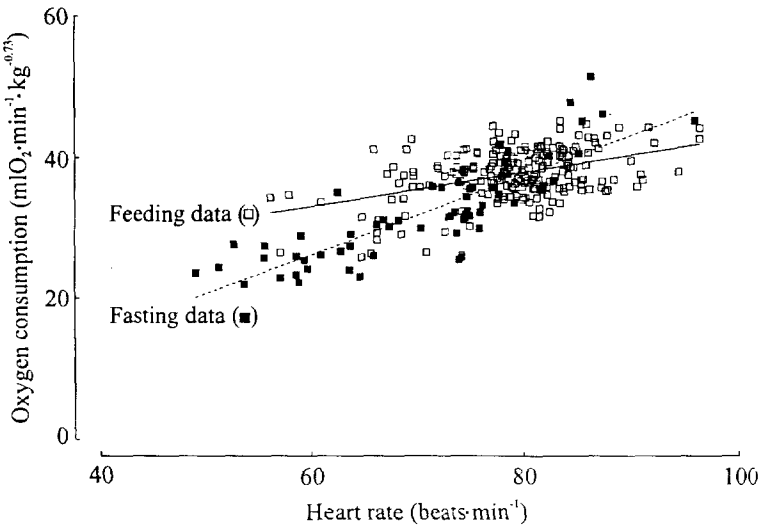

Fig. A4.4. Relationship between heart rate (beats·min^{-1}) and oxygen consumption (mlO_2·min^{-1}·kg$^{-0.75}$) as determined separately for feeding, $\dot{V}O_2 = (0.24 f_H \pm 0.03) + (18.49 \pm 2.68)$ ($r^2 = 0.19$), and fasting, $\dot{V}O_2 = (0.56 f_H \pm 0.04) - (7.43 \pm 3.27)$ ($r^2 = 0.71$), trials. Both regressions were significant at the $p<0.01$ level.

Discussion

A heat increment of feeding is commonly observed in vertebrates after ingestion of a meal. This is thought to be due to the biochemical oxygen-requiring mechanical processes occurring during digestion (Blaxter, 1989; Rosen and Trites, 1997). As expected, oxygen consumption of Male 2 continued to rise over all 3-hour feeding runs regardless of meal size, supporting the idea that digestion of food requires an increase in oxygen delivery.

Unlike metabolism, however, heart rate increased in only one 12 kg feeding run, and either remained unchanged or decreased over time during the other 12 and 6 kg feeding trials (Fig. A4.1, Table A4.1). Various mammalian studies, predominantly on humans, have shown postprandial increases in heart rate, stroke volume, blood pressure, and/or cardiac output following feeding (Grollman, 1929; Gladstone, 1935; Muller et al., 1992; Sidery and MacDonald, 1994; Kearney et al., 1995), even when exercise effects were considered (Yi et al., 1990). One study, however, reported no change in such variables after feeding (Jones et al., 1965). Unlike many human studies where the haemodynamic response may continue for hours, haemodynamic increases did not extend beyond the cessation of feeding in dogs, calves, and pigs, but returned to baseline values at the end of feeding (Fronek and Stahlgren, 1968; Houpt et al., 1983; Kelbaek et al., 1989).

In those studies where cardiac output increased postprandially, there is also considerable conflicting data concerning whether increased stroke volume or heart rate is the major contributor. Concurrent with the heat increment of feeding, some studies have shown that heart rate and stroke volume contribute equally to postprandial cardiac output,

while in others, either variable may increase more than the other (Kelbaek et al., 1989; Waaler et al., 1991; Muller et al., 1992; Sidery and MacDonald, 1994). Thus, changes in metabolism may correlate with stroke volume, heart rate, or both. In young lambs, oxygen extraction appeared responsible for the increase in $\dot{V}O_2$, not stroke volume or heart rate (Grant et al., 1997).

In the cases where heart rate did not increase over time in the sea lion I studied, it is possible that stroke volume or oxygen extraction increased to compensate for the increased oxygen consumption during digestion. An alternative explanation, as suggested by Yi *et. al.* (1990), is that blood flow may have been redirected from other vascular beds in the body to the digestive organs. Thus, cardiac output would not have been elevated as a result of increased heart rate and/or stroke volume. Another possibility is that the oxygen reserves an animal uses for diving were not used entirely for this behavior but were also used at the surface to fuel the heat increment of feeding, such that an elevated heart rate was not needed to meet the increased oxygen demands (W. Milsom, pers. comm., Uinversity of British Columbia).

The sea lion in my study had short diving intervals (usually less than one minute), which supports the idea that oxygen reserves may not have been depleted and could have potentially been used for another purpose (i.e. digestive processes). However, aerobic metabolism can only be fueled by sea lions' oxygen stores for 2-8 minutes. Thus, over a long period of time, all the oxygen burned in the body's tissues will be taken from the respirometry dome. Since the gut does not have its own oxygen store, oxygen must be delivered by the heart pushing enough blood to it (R.D. Andrews, pers. comm.,

University of British Columbia). Without data on other haemodynamic variables and on oxygen reserves, such conclusions, however, are speculation only.

A question that arises from the behavior of heart rate in Male 2 is whether 3-4 hours was long enough for digestion to increase heart rate. An increase in oxygen consumption for all feeding trials was observed, clearly indicating that digestion was underway. This was consistent with a prior study by Rosen and Trites (1997) that followed the heat increment of feeding after feeding 2 and 4 kg meals to captive Steller sea lions of similar size to those in the current study. They recorded increases in oxygen consumption after 30 minutes and found oxygen consumption reached its peak between 3 and 4 hours after feeding and declined to baseline values 6-8 and 8-10 hours after the small and large meals, respectively. The larger meal postponed the peak by about 1 hour.

It is difficult to believe that if heart rate was to increase during digestion at all, that it would not be evident within the first 4 hours after ingestion, especially since oxygen consumption had risen significantly. Studies that have compared the effect of meal size on haemodynamic variables have shown that large meals will postpone the peak of the postprandial increase in cardiac output relative to smaller meals (Waaler et al., 199; Sidery and MacDonald, 1994). Whether the same effect is true of heart rate is unclear. However, cardiac output increased in these studies as a result of roughly equal increases in both heart rate and stroke volume, indicating that peak heart rate may have been postponed as well. Increases in cardiac output were evident within 30 minutes after feeding even for large meals, thus supporting the notion that heart rate increases should be observed within 4 hours, even with the larger meal sizes.

Although the heart rate of Male 2 did not increase with time in most feeding trials, mean heart rate was generally higher than when the animal was fasted and was swimming without a current (ANOVA comparing heart rate after 0, 6 and 12 kg food $p<0.001$, and Fig. A4.2). This higher heart rate appears consistent with previous mammalian studies. The exact mechanism for the increase in heart rate is unclear, but drops in postprandial total peripheral resistance (due to blood flow shifted to the gut) have been measured. One suggestion is that such a drop would unload peripheral baroreceptors leading to increased activity of sympathetic cardiac fibers. Reduced atrial filling and distension would increase vasomotor tone (Sidery and MacDonald, 1994). Fig. A4.2 also shows that larger postprandial increases in heart rate were associated with larger meal sizes, as has been reported by other studies varying meal size (Grollman, 1929; Waaler et al., 1991; Sidery and MacDonald, 1994). This effect of feeding on heart rate may result from the splanchnic area being more perfused after a large meal when compared to a small meal.

Regressing oxygen consumption on heart rate with data collected after 12 kg-feedings failed to produce a relationship. Data from only the 6-kg feedings provided only a weak relationship ($p<0.01$, $r^2 = 0.26$). Regressing $\dot{V}O_2$ onto f_H from all feeding trials (both 6 and 12 kg) yielded a poor relationship ($r^2 = 0.17$, $p<0.01$, Fig. A4.3) that was significantly different from the fasting regression in Chapter 1 (Fig. A4.4). Possible time lags were investigated by shifting either the heart rate or oxygen consumption data forward by 5, 10, or 15 minutes, but resulted in successively poorer relationships. The above findings indicate that digestion alters the $f_H/\dot{V}O_2$ relationship when compared to a fasting condition.

With my limited data, the effect of feeding on the relationship between f_H and $\dot{V}O_2$ cannot be clearly explained. Further study that includes looking at temporal effects and experimenting with a larger sample size with longer trials appears necessary. It may eventually be necessary to study feeding while swimming against various water current speeds to further elucidate the behavior of the feeding regression at the higher levels of oxygen consumption and heart rate that are experienced in the wild.

Conclusion

My feeding experiment showed that, while digestion of food raised the metabolism of Steller sea lions, it failed to invoke a corresponding rise in heart rate. This suggests that regression models derived from fasted individuals will underestimate metabolism from measurements of heart rate of free-ranging sea lions. Further research to clarify the effects of feeding on the relationship between f_H and $\dot{V}O_2$ is warranted before metabolism can be accurately estimated from heart rate when sea lions are feeding in the wild.

Summary

Some studies of captive pinnipeds and other species have shown that the metabolic rate of fasted individuals can be estimated from heart rate. Although some researchers have suggested that this method can be used to estimate the metabolic rate of free-ranging pinnipeds, it has not yet been determined whether the relationship between heart rate and metabolism is altered by cardio-respiratory and metabolic adjustments to feeding. I sought to test whether digestion affects this relationship by feeding a captive male Steller sea lion either 6 or 12 kg of herring prior to swimming for 3 hours in a swim mill while both heart rate (f_H) and oxygen consumption ($\dot{V}O_2$) were monitored. Three trials were run for each amount of food. Oxygen consumption, measured with open circuit respirometry, increased over time in all six trials after ingestion of the meal. However, heart rate, measured with subcutaneous electrodes, increased in only one of the 12-kg meals. The relationship, $\dot{V}O_2 = (0.05 f_H \pm 0.01 \text{s.e.}) - (4.73 \pm 0.64)$ ($r^2=0.17$, $p<0.01$), that followed feeding was significantly different from that derived in a previous study while the animal fasted, $\dot{V}O_2 = (0.14 f_H \pm 0.01) - (1.79 \pm 0.80)$ ($r^2=0.70$, $p<0.01$). This suggests that feeding alters the relationship between f_H and $\dot{V}O_2$, and that alternating fasting and feeding intervals must be taken into account if heart rate is to be used to estimate energy expenditure of free-ranging sea lions or other species.